Post-transplant outcome of ovarian tissue cryopreserved after chemotherapy in hematologic malignancies

Fertility preservation is part of the management of patients with hematologic malignancies because of the gonadotoxicity of treatments such as myeloablative hematopoietic stem cell transplantation conditioning regimens, resulting in premature ovarian failure and infertility.

Ovarian tissue cryopreservation (OTC) has become a fertility preservation technique commonly offered to patients.[1] The first birth after ovarian tissue transplantation (OTT) into the pelvis was reported in 2004 in a woman previously treated for Hodgkin lymphoma.[2] So far, more than 130 births have been reported worldwide after autologous OTT.[3] In some countries OTT is now considered routine.[4]

Ovarian tissue cryopreservation has mostly been performed before exposure to chemotherapy, but the safety regarding ovarian function subsequent to antitumor treatment administration before OTC remains a matter of debate.[5] The Edinburgh selection criteria for OTC do not consider patients older than 15 years who had previously received chemotherapy.[5] Indeed, quantitative alterations in the number of oocytes depend on the type of chemotherapy and the age of the patient at the time of treatment. It has been shown that chemotherapy increases the rate of nuclear abnormalities in granulosa cells and of vacuolization in oocytes. It also generates vascular alterations and ovarian fibrosis.[6,7] These abnormalities could not be related to a particular type of chemotherapy.

However, there are many situations in which fertility preservation in patients with hematologic malignancies cannot be performed before the initiation of the chemotherapy. These include patients with severe neutropenia and/or coagulation disorders at diagnosis, patients requiring immediate treatment or patients initially treated with chemotherapy with low gonadotoxic risk in whom a relapse or progression calls for some other chemotherapy with a higher gonadotoxic risk.[8] Restoration of ovarian function after transplantation of ovarian tissue cryopreserved after the beginning of chemotherapy has been reported in a few cases. The first birth after transplantation of ovarian tissue previously exposed to chemotherapy was reported in 2005.[9] In 2016, the same team reported on ten patients previously exposed of whom 40% had had at least one child after OTT with a mean follow up of 3.18 years.[10] Determining in a large series of patients whether chemotherapy received before OTC could have a negative impact on ovarian function after transplantation of ovarian tissue remains a major issue.

We report here on 25 patients who had been treated by chemotherapy for hematologic malignancies before OTC, who wished to become pregnant after the cure of

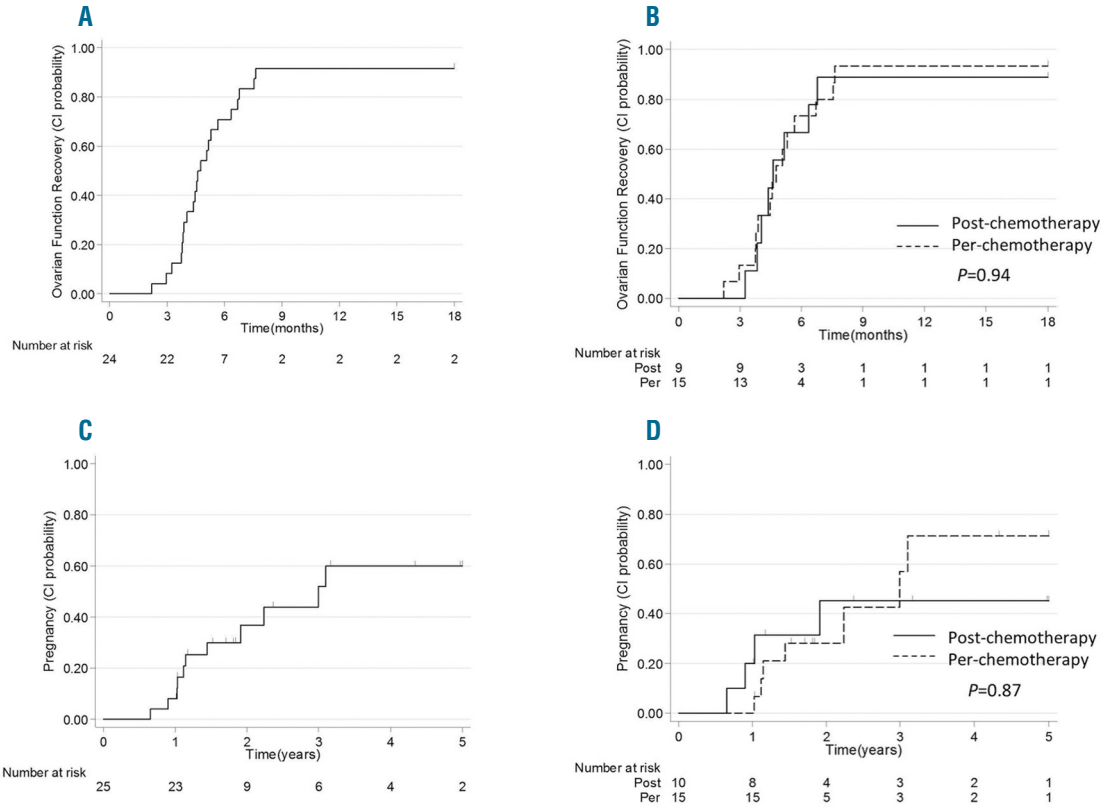

Figure 1. Ovarian function recovery and pregnancy after ovarian tissue transplantation. Cumulative incidence of ovarian function recovery related to total population (A) and if ovarian tissue cryopreservation was carried out post- or per-chemotherapy (B). Cumulative incidence of pregnancy related to total population (C) and if ovarian tissue cryopreservation was carried out post- or per-chemotherapy (D).

their disease, and who underwent an orthotopic OTT. Our study demonstrates the efficacy of OTT in this setting.

Our OTT program had been approved by the ethical committee of the Pitié-Salpêtrière Hospital. Appropriate consent forms were signed by all patients. Among these 25 patients, 16 were diagnosed with Hodgkin lymphoma, 7 with non-Hodgkin disease, and 2 with acute myeloid leukemia (AML) (patient characteristics are summarized in Table 1).

Ovarian tissue cryopreservation was planned as soon as a highly gonadotoxic treatment was decided. Cryopreservation was performed after the patient reached complete remission to reduce any intra-ovarian tumor infiltration. The ovarian tissue was collected under laparoscopy. Once the ovarian tissue had been removed,

Table 1. Characteristics of patients at the time of ovarian tissue cryopreservation, treatments received before and after ovarian tissue cryopreservation and outcomes of ovarian tissue transplantation.

Patient n.	Pathology	Age (years)		Treatment received before OTC	Treatment received after OTC	OTT outcome
		OTC	OTT			
POST-CHEMOTHERAPY						
1	HL	27.1	37.2	6 x VEBED	Autologous HSCT	1 birth
2	HL	24.4	31.4	3 x ABVD	Autologous and allogeneic HSCT (TBI 2Gy)	1 birth
3	HL	27.8	37.8	6 x ABVD	Autologous HSCT (TBI 6 Gy)	OFR
4	HL	31.6	36.5	6 x EBVP	Autologous HSCT	1 biochemical pregnancy, 1 birth
5	HL	27.0	32.2	6 x ABVD	Autologous HSCT	1 birth
6	HL	30.9	37.7	2 x ABVD, 4 x BEACOPP	Autologous HSCT	OFR
7	HL	28.1	32.8	4 x BEACOPP, 4 x BEACOPPesc, 3 x DHAP, Autologous HSCT**	Allogeneic HSCT (TBI 2Gy)	OFR
8	HL	19.9	24.1	8 x EBVP, 3 x MOPP	Autologous HSCT (TBI*)	OFR
9	HL	18.8	29.7	6 x ABVD	Autologous HSCT	NOFR
10	NHD	26.4	34.4	7 x R-CHOP, Autologous HSCT**	2nd Autologous HSCT	OFR
PER-CHEMOTHERAPY						
11	HL	18.6	24.6	2 x VBVP, 2 x MOPP, 2 x OPPA	Autologous HSCT	2 births, 1 miscarriage
12	HL	30.7	33.9	2 x ABVD, 1 x BEACOPPesc	Autologous HSCT	OFR
13	HL	18.9	26.7	ABVD	Autologous HSCT	OFR
14	HL	23.8	31.4	6+4 x ABVD	Autologous HSCT	1 birth
15	HL	31.7	37.7	4 x ABVD, 2 x MINE	Autologous and allogeneic HSCT	OFR
16	HL	21.0	28.6	4 x ABVD	Autologous HSCT	1 miscarriage after ART, 1 ongoing pregnancy after ART
17	HL	27.9	35.5	4 x ABVD	Autologous HSCT (TBI 10Gy)	1 abortion, 1 birth, 1 miscarriage
18	NHD	21.3	27.2	1 x CHOP	Allogeneic HSCT	1 ongoing pregnancy
19	NHD	31.9	34.4	6 x R-CHOP, 3 x R-ESHAP	Autologous HSCT	NOFR
20	NHD	29.8	40.4	4 x ACVBP	Autologous HSCT	OFR
21	NHD	16.6	28.7	High-dose chemotherapy including cyclophosphamide x2 several Vinblastine infusions	Autologous and allogeneic HSCT (TBI*)	1 birth
22	NHD	25.9	29.0	1 x ABVD	Autologous HSCT	2 miscarriages, 1 birth, 1 ongoing pregnancy
23	NHD	29.5	31.3	1 x ACVBP	Autologous and allogeneic HSCT	OFR
24	AML	26.5	37.2	2 x ADE	Allogeneic HSCT (TBI*)	OFR
25	AML	19.0	27.7	1 x MAC, 1 x HDAC+ASPA	Allogeneic HSCT	OFR

ART: assisted reproductive technology; biochemical pregnancy: β-hCG under 100 mIU/mL and no sign of clinical pregnancy; HL: Hodgkin lymphoma; HSCT: hematopoietic stem cell transplantation; NHD: non-Hodgkin disease; NOFR: no ovarian function recovery; OFR: ovarian function recovery; OTC: ovarian tissue cryopreservation; OTT: ovarian tissue transplantation; TBI: total body irradiation. *Unknown dose. **Despite gonadotoxic treatment before OTC, residual ovarian function was considered compatible with fertility preservation. Treatments: ABVD: doxorubicin, bleomycin, vinblastine, dacarbazine; ACVBP: doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone; ADE: daunorubicine, aracytine, etoposide; BEACOPP and BEACOPPesc: bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone; EBVP: epirubicin, bleomycin, vinblastine, prednisone; HDAC+ASPA: aracytine; L-asparaginase; MAC: aracytine, mitoxantrone; MINE: mitoguazone, ifosfamide, navelbine, etoposide; MOPP: mechlorethamine, vincristine, procarbazine, prednisone; OPPA: vincristine, prednisone, procarbazine, doxorubicine; R-CHOP: rituximab, doxorubicin, cyclophosphamide, vincristine, prednisone; R-ESHAP: rituximab, cisplatin, etoposide, cytarabine, methylprednisolone; VBVP: vinblastine, bleomycin, etoposide, prednisone; VEBED: vinblastine, epirubicin, bleomycin, etoposide, prednisone.

it was placed in a sterile transport medium (FerticultTM HEPES, Fertipro, Beernem, Belgium) and transported on ice to the laboratory for freezing and cryopreservation. Most of the ovarian medulla tissue was removed and the ovarian cortex was split into fragments. Each fragment was placed into a cryoval containing 1 mL of freezing solution. Dimethylsulfoxide (Braun Medical SA, Boulogne-Billancourt, France or WAK-Chemie Medical GmbH, Steinbach, Germany) was used as the cryoprotectant and a slow cooling protocol was implemented.

At the time of OTC, the patients were either some time (> 3 months) from their last chemotherapy ("post-chemotherapy" group, n=10, 40%) and ovarian sampling was carried out before resuming chemotherapy, or were undergoing chemotherapy ("per-chemotherapy" group, n=15, 60%) for progression of the disease or for emergency treatment. No per-chemotherapy patient received LHRH analogs before or per OTC. This information was not recorded for post-chemotherapy patients. Indication of ovarian cryopreservation was autologous (n=17), allogeneic (n=4), or autologous and allogeneic hematopoietic stem cell transplantation (n=4). In seven patients, the conditioning regimen included total body irradiation (TBI).

All OTT were carried out after approval from the hematologist and after checking the absence of tumor contamination in a fragment of ovarian cortex by histological study. For the two patients who had AML and were in complete remission at the time of OTC, a negative minimal residual disease (MRD) was assessed by molecular biology in one of the randomly selected frozen ovarian fragments (MLL-AF4 and *RUNX1* mutation as MRD targets).

On the day of OTT, the ovary fragments were thawed according to a rapid thawing protocol and placed in successive baths of thawing solution, with decreasing concentrations of dimethylsulfoxide. The ovarian tissue was transplanted by laparoscopy. The fragments were grafted orthotopically in two sub-peritoneal pouches created in the ovarian fossa. Thawed ovarian fragments were attached to one another and placed in the transplant sites. After OTT, the ovarian function was monitored each month by measuring FSH, LH, estradiol and AMH to assess the recovery of normal levels of gonadotropins and renewed secretion of estradiol by the transplant, and by trans-vaginal ultrasound to assess follicular growth in the ovarian transplants. The criterion used to define complete recovery of ovarian function was the occurrence of menstruation. Pregnancy was assessed by β-hCG blood test.

Cumulative incidence of ovarian function recovery (OFR) was defined as the time between transplantation and menstruation recovery, censoring patients with no recovery at the last follow up. The cumulative incidence of pregnancy was measured by the time between transplantation and first pregnancy, censoring patients who were not pregnant at the last follow up. Comparisons between the two subgroups were performed with an univariate Cox model (STATA 12.0 Corporation, College Station, TX, USA).

The median age of patients at the time of OTC in our cohort was 26.5 years (range, 16.6-31.9), and was similar to that in previously reported cohorts.[10,11] There was no difference in median age between the post-chemotherapy group (27.1 years; range, 18.8-31.6) and the per-chemotherapy group (25.9 years; range, 16.6-31) (*P*=0.62). The median time between OTC and OTT was 6.8 years. The median follow up after ovarian transplantation was 32 months.

At the time of OTT, all patients except one were under premature ovarian failure. This particular patient was not included in the cumulative incidence evaluation of ovarian function recovery. The cumulative incidence of ovarian function recovery at one year was 92% (95%CI: 77% to 99%) with a median time to recovery of 4.6 months (range, 2.2-7.6). No difference was found between post-chemotherapy patients [cumulative incidence 89% (95%CI: 61-99%)] and per-chemotherapy patients [cumulative incidence 93% (95%CI: 74-99%)] (*P*=0.94) (Figure 1A and B). These results compared favorably with those reported in patients who had not received chemotherapy before ovarian cryopreservation, showing a 1-year recovery of ovarian function in 67% of patients.[11]

In our study, the cumulative incidence of pregnancy was 52% (95%CI: 31-77%) at three years and 60% (95%CI: 37-83%) at five years, with no significant difference between post and per-chemotherapy groups [3-year cumulative incidence of pregnancy: 45% (95%CI: 19-81%) *vs.* 57% (95%CI: 28-88%), respectively, (*P*=0.87)] (Figure 1C and D). In the whole cohort, 11 patients became pregnant at least once (41% of all patients and 46% of patients who recovered ovarian function) and gave birth to at least one healthy child (n=8) or had an ongoing pregnancy (n=3). These pregnancy rates are similar to those published by Meirow *et al*. (40%) in their series of ten patients exposed to chemotherapy prior to ovarian preservation.[10] Interestingly, the rate of women who became pregnant at least once was 32.6% in the series of 49 patients who had an ovarian tissue cryopreservation before chemotherapy.[11]

To our knowledge, this study is the largest of its kind and suggests that the results of OTC are not affected by whether the patient receives or does not receive chemotherapy prior to cryopreservation. Moreover, from a practical point of view, a recent exposure to chemotherapy (< 3 months) does not modify the chances of recovering ovarian function and becoming pregnant.

In conclusion, in malignant hematologic diseases where starting chemotherapy may compete with early fertility preservation, ovarian tissue cryopreservation performed at any time, including after the start of chemotherapy, appears efficient and should be the preferred technique. The alternative (cryopreservation of mature oocytes after initiation of chemotherapy) is really not very efficacious.[12] To preserve fertility even after initiation of chemotherapy may allow a larger number of patients to become candidates for OCT and thus maintain their chances of motherhood. This is indeed a major factor that improves the quality of life of cancer survivors.[13] Our results may be regarded as a very important part of the information given to the patients with hematologic malignancies.

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