Detection of Non-Hodgkin's Lymphoma in ovarian cortex pieces during the process of cryopreservation

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Received: 22 April 2014 / Accepted: 2 July 2014 / Published online: 15 July 2014
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Introduction

Innovations in chemotherapy treatments are leading to increased survival rates among lymphoma patients. Non Hodgkin's Lymphoma (NHL) is the most common hematologic malignancy and one of the most common cancers in adolescents and young adults. The incidence of NHL has increased 2-fold over the past twenty years. Chemotherapy regimens for NHL always include cyclophosphamide, which is well-known to be gonadotoxic in an age- and dose-dependant manner [1–4].

The incidence of premature ovarian failure in case of NHL is about 5 % after CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), 14 % after hyper-CVAD (cyclophosphamide,

vincristine, adriamycine, dexamethasone) and 70–100 % after hematopoietic stem cell transplant (HSCT), with pregnancy rates of 50 %, 43 % and less than 5 %, respectively [5–7].

A large retrospective survey of 37 362 patients revealed that only 0.6 % of those patients conceived after autologous or allogenic stem cell transplantation [8]. In this respect, information about chemo-induced infertility in haematological malignancy needs to be given and fertility preservation options should be discussed with patients before treatment [7, 9, 10].

Ovarian cryopreservation is currently required when the risk of infertility is more than 50 % [11] and/or when chemotherapy cannot be delayed sufficiently enough for performing mature oocyte cryopreservation after controlled ovarian hyperstimulation [12].

Over the last decades, about 60 patients underwent cryopreserved ovarian tissue auto-transplantation, in the purpose to restore fertility after a gonadotoxic treatment, resulting in 24 live births [13]. Pregnancies were obtained after orthotopic transplantation and occurred either spontaneously or after IVF [13, 14]. Hodgkin Lymphoma (HL) and Non Hodgkin Lymphoma are common indications of ovarian cryopreservation [10, 13, 14]. To date, fifteen auto-transplantations have been performed for HL and six for NHL respectively without any disease recurrence [13]. Nevertheless, Bittinger et al. recently showed an ovarian involvement of a stage III HL within the ovarian cortex pieces systematically assessed for the presence of tumor cells before storage [15]. We currently report the histological detection of NHL cells within the ovarian tissue removed for subsequent cryopreservation in a 24-year-old woman diagnosed with a Primary Mediastinal large B-cell Lymphoma.

Material and methods

A 24-year-old woman without significant medical history consulted for a superior cava venae syndrome. A NHL with

Capsule Recent data regarding the potential ovarian involvement by lymphoma cells urge caution when a reimplantation of ovarian tissue is planned. Patients have to be rigorously informed about this risk before the decision of ovarian removal or auto-transplantation.

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supraclavicular and axillary location was suspected following a supraclavicular lymph node's biopsy during the exploration of this superior cava venae syndrome. Positron Emission Tomography with 18 F-fluoro-2-deoxy-D-glucose (FDG), which is presented in Fig. 1, confirmed an intense uptake of the mediastinal lymph nodes, associated with a nodular splenic lesion. A slight uptake is also described in the left pelvic area (Fig. 1). The bone marrow examination did not show evidence of tumor involvement. The clinical stage was III with an international prognosis index of 2. Given these unfavourable features, the patient required an urgent chemotherapy regimen that included alkylating agents with the CVAD protocol (Doxorubicine 300 g/m², Cyclophosphamide 4 800 mg/m², Vinblastine 16 mg/m², Bléomycine 80 mg/m² and Prednisone) associated with the monoclonal antibody anti-CD20 (Rituximab®). Consequently, the patient was urgently referred to our fertility preservation unit in order to examine the indication of ovarian tissue harvesting. The patient was nulliparous and normally cycling. Her pre-treatment ovarian reserve was assessed through AMH levels and transvaginal ultrasonographic assessment of the antral follicle count (AFC). The serum AMH level was of 32 pmol/l and the total number of antral follicles was 24. The patient gave her informed consent regarding ovarian removal for cryopreservation under laparoscopic guidance. She also gave her informed consent to be enrolled in a follow-up of her ovarian reserve marker during and after chemotherapy by performing serial AMH and AFC measurements for 2 consecutive years according to our previously-reported protocol [16].

Results

Thirty-two small pieces were excised from the removed ovary in order to be cryopreserved. Four of them were isolated for subsequent histological analysis as a routine protocol in our department. All of this collected tissue showed evidence of

Fig. 1 Slight uptake in the left pelvic area at the positron Emission Tomography with 18 F-fluoro-2-deoxy-D-glucose

ovarian involvement by lymphoma cells, despite normal macroscopic aspect (Fig. 2). Final histological results of the lymph node were obtained the day after cryopreservation and were consistent with the diagnosis of Primary Mediastinal Large B-Cell Lymphoma (PMBCL).

After the four R-CVAD cycles, the patient received a BEAM protocol (BCNU 300 mg/m², etoposide 100 mg/m², cytarabine 200 mg/m², melphalan 140 mg/m², procarbazine 100 mg/m², prednisone 40 mg/m²) prior to perform a stem-cell transplantation.

Two years after the end of treatment, the patient remains in remission. She recovered only three menstrual cycles during the last two years. AMH levels remained undetectable with high FSH levels. Fortunately, despite this poor reproductive prognosis, she conceived spontaneously and delivered a healthy baby.

Discussion

Ovarian tissue cryopreservation is theoretically the most efficient way of preserving thousands of ovarian follicles at one time in term of pregnancies. This technique of fertility preservation remains the only option for prepubertal females and for those women who cannot delay their cancer treatment in order to undergo ovarian stimulation for egg retrieval. Nevertheless, despite these positive aspects and the increasing number of live births after successful ovarian tissue transplantation, ovarian cryopreservation still should be considered experimental [17]. Indeed, there is concern regarding the possible presence of malignant cells in the ovarian tissue, which could lead to recurrence of the primary disease after reimplantation [7, 10, 18, 19]. Hence, it remains difficult at present to establish the imbalance between the real chances of pregnancy this technique can offer, versus the risk of mainly reintroducing the disease and also reducing the ovarian reserve.

HL and NHL are frequent indications for ovarian tissue freezing and are commonly considered at minimal risk of

Fig. 2 Massive involvement of NHL cells in the ovarian cortex

ovarian involvement [18, 20, 21]. However, caution is required due to the results of two studies [22, 23]. On one hand, Shaw et al. reported that ovarian tissue collected from AKR mice with lymphoma could transfer the disease to healthy recipient animals [22]. And on the other, Kyono et al., in a study aiming to evaluate the feasibility of auto-transplantation based on the analysis of 5 571 autopsy findings of young females (but dead from malignant diseases!), showed 4.3 % and 9.8 % of ovarian involvement by HL or NHL cells, respectively [23]. Few experimental studies have tested the human ovarian tissue from both HL or NHL and failed to show evidence of ovarian involvement by lymphoma cells [18, 20, 24, 25]. To date, 15 auto-transplantations have been reported in HL patients and 6 in NHL without any disease recurrence, knowing that the follow-up after auto-transplantation in one of the HL patients is now up to 8 years [19, 26–34]. Nevertheless, a recent case-report showed an ovarian involvement in the histologic examination of ovarian cortex pieces in a stage III HL [15]. The transvaginal ultrasonographic exam and the Positron Emission Tomography with 18 FDG performed before the surgery didn't detect any abnormality in this case.

In the current case-report, the patient was diagnosed a rare subtype of diffuse large B-cell lymphoma, whose frequency is estimated at 2–5 % of non-Hodgkin lymphoma [35]. The epidemiological features are at an early age and a sex ratio to 2 in favor of females, with no specific identified risk factors so far. Compression of large mediastinal vessels is common in patients with PMBCL [36]. Although ovarian locations represent a rare event in Hodgkin and non-Hodgkin lymphoma, it seems that extra nodal localisations are more frequent in PMBCL subtype. Caution is thus required before the auto-transplantation of frozen-thawed ovarian tissue in this context. In a recent publication, according to recent reports, Dolmans et al. adapted the classification of Sonmezer and Oktay [21] by recategorizing NHL from low to moderate risk of ovarian involvement [19]. Indeed, this Belgian team detected malignant cells by histologic evaluation in 2 patients from 32 (6 %) with NHL [19]. The real presence of malignant cells in the ovarian tissue is surely under-estimated because the histologic evaluation and immunophenotyping B-cells are well-recognized as not being sufficiently sensitive.

In our case report, the Positron Emission Tomography with 18 FDG highlighted a slight uptake in the pelvic area that we have not taken into account in our decision of ovarian cryopreservation. Indeed, it could have been an a-specific uptake related to a functional cyst. As we didn't have time to harvest mature oocytes, the ovarian cryopreservation was the only option for this patient. The question is then asked as whether it would be better to perform the cryopreservation after first lines of chemotherapy in order to decrease the risk of cancer recurrence after auto-transplantation. Ovarian cryopreservation aims to preserve primordial follicles, which are

presumably less damaged by chemotherapy than growing follicles [4]. Meirow et al. reported a live birth after autotransplantation of ovarian tissue that had been cryopreserved after a first line of chemotherapy [37]. But we have to keep in mind that chemotherapy regimens damage the ovaries through apoptotic effects but also through cortical fibrosis and blood microvessels injury [38]. One of the consequences of this vascular toxicity is that the duration of the restored ovarian function after auto-transplantation could be shorter (1–2 years) when patients had undergone chemotherapy before cryopreservation [38].

New developments for the reutilization of the harvested ovarian tissue, all experimental, are emerging. One approach is the re-implantation of isolated follicles from the ovarian tissue [39]. Indeed, isolated human preantral follicles can survive and grow in vitro, that represents a lot of hope for the future [40–43]. Another approach is the In Vitro Maturation (IVM) of immature oocytes [44–46]. Last, combined procedure, meaning the combination of IVM of immature oocytes and ovarian tissue cryopreservation on the same day, could be a promising strategy too [12, 44–46].

In conclusion, recent data regarding the potential ovarian involvement in HL and NHL urge caution when a reimplantation of ovarian tissue is planned. This case report stresses the need to discuss it with patients, for both the decision of ovarian removal for cryopreservation and the decision of auto-transplantation. It is crucial to make sure that women and reproductive doctors will be obviously both helped in their decision by a multidisciplinary approach [47].

Conflict of interest No conflict of interest.

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