

Fertility preservation in young leukemia patients

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Leukemia forms the most common type of childhood cancer and may also occur during adolescence and through reproductive years. It accounts for 31% of cancers in young girls, 12% of cancers in female adolescents and 3% of cancers in adults (females <40 years) [1]. Looking at the past four decades, a dramatic increase in leukemia survival rates has occurred, especially among children [2]. For example, improved treatment for childhood ALL increased the 5-year survival rate from 57% between 1975 and 1979 to 90% between 2003 and 2009 [3], and in current times, 10-year survival rates are expected to exceed 80% [4]. Leukemia patients are frequently treated with aggressive gonadotoxic regimens which expose them to possible iatrogenic premature ovarian failure and resultant sterilization. Together with a young age at diagnosis and the prospect of favorable prognosis, this makes fertility preservation an important element of the management of these patients. However, fertility preservation for leukemia patients is highly challenged by considerations specific for their disease such as prepuberty, poor medical condition on presentation and the need for urgent chemotherapy, all of which may preclude the use of conventional assisted reproductive technologies. A restrictive approach towards utilizing cryopreserved ovarian tissue due to the risk for malignancy reintroduction on transplantation, further minimize the options that currently exist for these patients. Additionally, the clinical presentation of leukemia

patients gives rise to distinct concerns which should be taken into consideration. For example, in the presence of thrombocytopenia, administration of clotting factors and platelets prior may prior to invasive fertility preservation procedure should be considered.

Reproductive damage

According to the American Society of Clinical Oncology, the risk of permanent amenorrhea following standard AML therapy or multiagent acute lymphoblastic leukemia (ALL) therapy is less than 20% [5, 6]. Gonadotoxicity of chemotherapeutic regimen depends on gonadotoxic contribution of each chemotherapy agent, number of cycles and total cumulative dose. It is well established that alkylating agents are responsible for the highest age-adjusted odds ratio of ovarian failure rates, in a dose-dependent manner [7-9]. Age at exposure to chemotherapy also plays a major role in determining the extent of ovarian damage, as older patients are more inclined to experience chemotherapy induced ovarian failure [10].

ALL treatment- The treatment of childhood ALL usually consists of 4-6 week of induction chemotherapy, which commonly involves the administration of vincristine, daunorubicin, pegylated L- asparaginase and methotrexate in cases of CNS involvement. Following induction therapy, most patients achieve complete remission and continue to consolidation therapy, which aims to eradicate the submicroscopic residual disease. Consolidation protocols vary in length and intensity, with some

involving the administration of 6-mercaptopurine, cytarabine and cyclophosphamide [11]. In general, induction and consolidation regimens for leukemia are reported to be associated with a low risk for ovarian failure. However, certain protocols do contain cyclophosphamide at higher doses. For example, patients treated with the GMALL protocol are administered with a total of 5.4-7.2 gram of cyclophosphamide [12]. Such cumulative dose causes definite ovarian damage and put them at an increased risk for future ovarian insufficiency [7]. The final stage of treatment in childhood ALL is maintenance which usually includes 6-mercaptopurine together with methotrexate, given for a period of 2 to 3 years.

AML treatment- the standard treatment of AML includes an induction therapy followed by consolidation, both of which are generally safe in terms of ovarian toxicity. Induction protocols mostly consist of anthracycline and extended exposure to cytarabine, occasionally with an additional third drug, such as etoposide. Consolidation protocols are based on high dose cytarabine and are usually given for several months.

CML treatment - specific targeted treatments, such as imatinib and other tyrosine kinase inhibitors, are currently the mainstay of treatment. To date, imatinib is not considered to impair the ovarian reserve in humans, and animal studies have failed to associate it with infertility [13, 14]

Hematopoietic stem cell transplantation (HSCT)- a non-neglectable number of leukemia patients necessitate HSCT. In the united states, from 2010 and on, more than one thousand ALL patients a year were treated with allogeneic HSCT [15], and recent clinical trials for pediatric disease reveal allogeneic HSCT use in 11-29% of enrolled AML patients [16]. In brief, HSCT is offered to patients who do not achieve remission

following induction therapy or those who experience recurrence following remission. Patients whose leukemia has high risk characteristics at diagnosis may be candidates for HSCT already at first remission. Conditioning treatments prior to HSCT, whether inclusive of total body irradiation or not, contain high dose alkylating agents and/or busulfan. Current guidelines quote over an 80% risk for permanent amenorrhea in women treated with HSCT [6]. It is therefore that in the case of planned HSCT, fertility preservation is undoubtedly warranted.

Fertility preservation measures

GnRH analogue- GnRH analogue is presumed to decrease follicular recruitment by inducing a hypogonadotropic state which exerts a positive effect on ovarian follicles pool. While there's a growing evidence to support its use in breast cancer patients [17, 18], this is not the case when hematologic malignancies [19, 20] or HSCT [21] are considered. GnRH-a alone should therefore not be relied on to preserve fertility but may serve as a mean to prevent menorrhagia in patients with thrombocytopenia secondary to chemotherapy [22].

Mature oocytes collection- Ovarian stimulation followed by mature oocytes collection can be performed in any IVF center, with both embryo and oocyte cryopreservation being considered as standard options for fertility preservation in present days. However, immediately after being treated with chemotherapy and at least 6 months ahead, oocyte collection becomes irrelevant. At this stage, decreased or no response to ovarian

stimulation can be expected [23]. More importantly, recent exposure to chemotherapy may result in genetically abnormal oocytes and adverse reproductive outcome such as high abortion and malformation rates [23, 24]. A recent study has found a clinical pregnancy rate of 40.8% among oncologic patients who used their vitrified oocytes[25]. Such encouraging data should be viewed judiciously, as it originates from centers with a vast experience in oocyte freezing. An important observation that can be drawn from the same study is that leukemia patients formed only 1.2% of those undergoing oocyte cryopreservation. This probably reflects the unfeasibility of oocyte collection in prepubertal girls, lack of time for ovarian stimulation due to poor medical condition and the need for urgent cancer treatment.

Immature oocytes collection -immature oocytes can be retrieved at any time during the menstrual cycle, both during the follicular and the luteal phase, with no significant difference in maturation and fertilization rates [26, 27]. This forms a favorable feature when tight schedule is dictated by oncologic considerations. While in conventional ART immature oocytes are collected transvaginally, a combined procedure of IVM and OTCP is commonly done for fertility preservation purposes, where oocytes are often collected ex-vivo. The latter is highly relevant for young leukemia patients for whom transvaginal aspiration is occasionally not practical. Importantly, as in the case for mature oocytes, recent chemotherapy precludes the collection of immature oocytes, whether performed ex-vivo or in-vivo, yet again making IVM irrelevant for many leukemia patients. Moreover, it should be noted that in terms of pregnancy rates, the yield of immature oocytes collection during ovarian tissue harvesting is low [28], and only a few live-births

have been reported worldwide to occur following ex-vivo aspiration of immature oocytes [29, 30].

Ovarian tissue cryopreservation (OTCP)- With an estimated live birth rate of over 30% [31, 32] leading to the birth of more than 130 newborns worldwide [31], it is of no wonder that OTCP and transplantation are increasingly applied. Although leukemia itself represents a common indication for OTCP [33, 34], re-transplantations are generally avoided in most fertility preservation centers due to an estimated high risk for malignancy reintroduction on transplantation [35].

- **Residual disease and risk for disease reintroduction-** In recent years, several small studies have focused on tests aiming to evaluate involvement of harvested ovarian tissue with Leukemia cells. In all studies combined, 4 out of 106 samples showed evidence for leukemia by histology. Out of 62 patients who had a known leukemia specific molecular, 36 showed evidence for minimal residual disease via PCR, which translates into a 58% rate of ovarian MRD [36]. The observed rates of ovarian MRD underline the possible risk that accompanies re-transplantation. However, as demonstrated in xenotransplantation studies, the neoplastic potential of ovarian MRD is difficult to predict, and its presence will not necessarily translate into disease recurrence following transplantation. In one xenografting study [37], immune deficient mice were transplanted with ovarian tissue collected from 18 leukemia patients. Sixteen of the patients had a known molecular marker which could be tested using PCR. None of PCR-negative and only a third of the PCR-positive grafts were found to induce leukemia when transplanted.

- **Timing of tissue harvesting-** In the aforementioned study, five out of 18 mice showed microscopic/macrosopic evidence for leukemia, all were transplanted with tissue harvested before patients' exposure to chemotherapy or after partial treatment with methotrexate. In a later study [38], 17 mice were transplanted with tissue collected during complete remission, none developed leukemia. The findings of these studies provide a motive to deliberately harvest while in complete remission, especially when both the graft's follicular density [38] and reproductive performance [32, 39] are not affected by pre-harvesting chemotherapy. To optimize harvesting timing, we follow the algorithm displayed on figure 1.
- **Limited clinical experience-** thus far only a single case of delivery following auto-transplantation in leukemia survivor has been described. In a recent report we have presented the case and the work-up performed to evaluate the patient's thawed tissue for the presence of leukemia cells. Tissue was tested for using light microscopy, cytogenetic analysis (FISH), next-generation sequencing (NGS), and xenotransplantation [40]. The lack of evidence for leukemia contamination in any of these tests, combined with a known complete remission on harvesting, were overall reassuring and favored transplantation. Starting 7 months following transplantation, controlled ovarian stimulation and IVF were performed. The patient conceived in the third cycle and delivered a healthy newborn. As of now, more than three years after transplantation, the patient is leukemia free. She has recently conceived spontaneously and gave birth to her second child.

Summary

An ongoing improvement in long-term survival rates and possible iatrogenic ovarian failure, especially in the context of HSCT, calls for incorporation of fertility preservation measures in the management of young leukemia patients. Due to a young age and the need for urgent chemotherapy on presentation, conventional ART is not applicable for most leukemia patients, which often makes OTCP their only chance for future biological offspring. This too is limited by an estimated high risk for leukemia reintroduction once transplantation takes place. In current days, additional measures for fertility preservation are under intense research for future clinical application. These include the development of a transplantable artificial ovary or in-vitro folliculogenesis, both of which may potentially overcome the risk for leukemia reintroduction on auto-transplantation. While additional safe fertility preservation techniques are awaited, OTCP and transplantation may be carefully considered for leukemia patients, in case harvesting was performed during complete remission and following a meticulous search for MRD within the graft.

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