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Running title: Uterine volume after HSCT

Title

Uterine volume is dramatically decreased after Hematopoietic Stem Cell Transplantation during childhood regardless of the conditioning regimen.

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Capsule:

Uterine volume is significantly decreased after Hematopoietic Stem Cell Transplantation regardless of the conditioning regimen.

Structured abstract:

Objective: To study the impact of hematopoietic stem cell transplantation (HSCT) on uterine volume of childhood acute leukemia (AL) survivor depending on age at HSCT and the type of myeloablative conditioning (MAC) regimen.

Design: Prospective cohort study

Exposure: A multicentric prospective national study compared uterine volume in a cohort of childhood AL survivor adult women treated with HSCT, matched 1:1 to control women.

Pelvic MRI scans included diffusion-weighted imaging sequences. Scans were centralized for a double-blinded reading by two radiologists.

Main outcome Measures: Uterine volume, uterine body-to-cervix ratio, and apparent diffusion coefficient (ADC).

Results: Mean age at HSCT was 9.1 ± 0.3 years with a mean follow-up duration of 16.4 ± 0.5 years. The cohort of 88 HSCT survivor women was composed of two sub-groups depending on the MAC regimen received: an alkylating agents-based regimen group (n=34) and a TBI-based regimen group (n=54). Among the 88 women, 77 were considered as having a “correct hormonal balance” with estrogens supplied by hormone replacement therapy (HRT) for premature ovarian insufficiency (POI), or thanks to a residual ovarian function. In the control group (n=88), the mean uterine volume was 79.7 ± 3.3 mL. Uterine volume was significantly decreased in all HSCT survivor women ($p < 0.0001$). After alkylating agents-based regimen, uterine volume was 45.3 ± 5.6 mL, corresponding to a significant volume reduction of 43.1 % [28.8 – 57.4] compared to the control group. After TBI, uterine volume was 19.6 ± 1.9 mL, corresponding to a significant volume reduction of 75.3 % [70.5 – 80.2] compared to the control group. After alkylating agents-based regimen, uterine volume was dramatically decreased in POI women without HRT compared to those with a correct hormonal balance (15.2 ± 2.6 Vs 49.3 ± 6 mL, $p < 0.05$). In contrast, after TBI, uterine volume was similar in all

women, with no positive effect of hormonal impregnation on uterine volume (respectively 16.3 ± 2.6 Vs 20.1 ± 2.2 mL).

Conclusion: Uterine volume was diminished after HSCT, regardless of the conditioning regimen. The physiopathology needs to be further investigated: specific impact of high dose of alkylating agent, impact of hormone deprivation around puberty, poor compliance to HRT, or different myometrial impact of HRT compared to endogenous ovarian estrogens?

Keywords

Uterus, hematopoietic stem cell transplantation, MRI, chemotherapy, total body irradiation

Introduction

With the improvement of survival rates, the reproductive outcome of childhood cancer survivors emerged as a main issue for their long-term quality of life (1). Hematopoietic stem cell transplantation (HSCT) is widely used to treat malignant and non-malignant hematological or immunological diseases, including the most severe forms of childhood acute leukemia (AL). In childhood AL, the choice of myeloablative conditioning (MAC) regimen before HSCT depends on the type of leukemia, the remission status and the graft source (2). Briefly, the commonly used MAC regimens are either chemotherapy plus total body irradiation (TBI-based regimen) or a combination of high-dose chemotherapy containing alkylating agents (alkylating agents-based regimen) (3).

The gonadotoxicity of MAC has been well reported in female, inducing a massive loss of ovarian reserve and fertility impairment after childhood cancer (4). TBI-based regimens induce radiation-related damage to both ovaries and uterus. Reduced uterine volume after childhood TBI has also been described, probably through radiation-induced fibrosis and uterine vasculature damage (5–7). Although spontaneous pregnancies are described after TBI-based regimens, the risk of adverse obstetrical outcome is high: spontaneous miscarriages, abnormal placentation, small for gestational age, preterm birth, low birth weight, risk of uterine rupture (8).

A few studies have observed smaller uterus after HSCT in women treated with chemotherapy only regimens (5,9,10). These studies suggested that alkylating agents could induce uterine damage as well as fibrosis and vascular damage to the ovarian stroma (11). Up until now, the impact of chemotherapy on myometrium and uterus has been poorly investigated (8).

The objective of our study was to assess the impact of HSCT on uterine volume of childhood AL survivors depending on the type of myeloablative conditioning (MAC) regimen and age at HSCT.

Materials and methods

Study population: the LEA cohort

The present study, named FERTILEA, is an ancillary study conducted on the LEA cohort. Briefly, the LEA (Leucémies de l'Enfant et de l'Adolescent) cohort is a French multicentric prospective cohort of more than 5 500 childhood acute leukemia long-term survivors (12). Since 2004, the objectives of the LEA cohort are to study the determinants (medical, socioeconomic, behavioral, and environmental) of medium- and long-term outcomes of patients treated for childhood acute leukemia (AL).

The multicentric prospective study FERTILEA was proposed to sixteen University Teaching Hospitals participating to the LEA program and was conducted in thirteen of them between November 2017 and June 2021. Eligible women to FERTILEA met the following criteria: (i) women > 18 years of age participating in the L.E.A cohort, (ii) who underwent HSCT during childhood or adolescence for AL (iii), after a MAC regimen including either TBI or a alkylating agents-based regimen, (iiii) who agreed with having a pelvic MRI to assess their uterine volume. A medical consultation with a physician specialized in oncofertility was proposed after the MRI to evaluate the ovarian function and estimate the observance of HRT in POI cases.

The present study FERTILEA included prospectively women from the LEA cohort with the main objective to assess uterine volume by MRI techniques. Data obtained from the pelvic MRI and the gynecologic assessment were prospective, but we also had retrospective data from the same women collected for 16 years in the context of the LEA program.

MAC regimens before HSCT

MAC regimen depended on protocols in use at the time of HSCT, underlying diseases and their status. Overall, 54 women received a total-body irradiation (TBI) containing regimen and another 34 an alkylating agents-based regimen. Most women prepared with TBI (47/54, 87%) were treated with a radiation regimen commonly used in France, i.e., a total dose of 12 Gy divided in 6 fractions (i.e. 2 Gy twice daily during 3 days) including lung shielding at 8 Gy. The other 7 women received a fractionated 10 to 11 Gy total dose. The cytostatic drugs used in association with TBI were: cyclophosphamide-only (n =30), cyclophosphamide and fludarabine (n =2), cytarabine and melphalan (n = 15) and etoposide (n =7). The mean calculated cumulative alkylating agents dose per patient, using the cyclophosphamide equivalent dose (CED) was 3 834 mg/m² (range: 0 to 11435) (13).

All but one of the 34 women from the alkylating agents-based regimen group were given busulfan (BU) at a myeloablative dose, one girl had melphalan instead. BU was given per os or IV in 16 divided doses (4 doses per day for 4 days) for a total dose of 16 mg/kg or 480 mg/m² when given per os. When prepared with a BU-containing regimen, patients also received cyclophosphamide (n = 24), melphalan (n = 4) or fludarabine (alone or in combination with thiotepa, n = 5). In the alkylating agents-based regimen group, mean cumulative CED dose was 11 217 mg/m² \pm 4461 (range: 4 235 to 29 470).

Control population

Each case was prospectively matched 1:1 according to age and parity to a control woman. The control group (n= 88) was composed of women enrolled in the radiology department of the principal investigator for a pelvic MRI during the study period, aged between 18 and 40 years, without any uterine pathology who underwent a pelvic MRI during the study period. Controls were excluded in case of uterine pathology such as adenomyosis or fibroids.

MRI scan

Pelvic MRI scans have been performed with a 1.5-T or 3 T magnetic resonance scanner, including diffusion-weighted imaging sequences. MR images were sent and centralized in the radiology department of the principal investigator. MRI scans contained a T2-weighted in all 3 planes (sagittal, coronal, and axial) and a Diffusion-weighted imaging (DWI) with apparent diffusion coefficient (ADC) maps in the axial plane. DWI is based on the intravoxel incoherent motion and has been used for the noninvasive quantification of the diffusion of water molecules in tissue, which is quantified by calculation of ADC. Knowing the variation of myometrial ADC according to genital activity and the known decrease in ADC values in fibrosis of other organs, we assumed that a decrease in ADC values could be inferred in women with uterine fibrosis (14,15). The following parameters were recorded: uterine volume measured on T2 weighted (anteroposterior x transverse x craniocaudal dimensions) x 0.52) and uterine body-to-cervix ratio. ADC values of the myometrium were measured on an ADC map by placing two regions of interest (ROI). We measured two ROIs and used the averaged value, rather than just one ROI, to reduce random variability in the measurements. All case data have been analyzed with a double-blinded reading by two independent radiologists, without knowing any information concerning the type of MAC regimen.

Study endpoints

The main study endpoint was the uterine volume. The secondary endpoints were uterine body-to-cervix ratio and ADC ($\times 10^{-3} \text{ mm}^2/\text{s}$).

In the HSCT group, we also collected data about type of acute leukemia, type of MAC, age at HSCT, pubertal status at the time of HSCT, hormonal status at the time of the study (premature ovarian insufficiency or spontaneous menstruations), HRT or not in case of premature ovarian insufficiency (POI), spontaneous pregnancy or pregnancy obtained after Assisted Reproductive Technology and follow-up (early miscarriage before 12 weeks of

gestation, late miscarriage between 12 and 22 weeks of gestation, live birth). POI was defined by a hypergonadotropic hypogonadism in women under 40.

In the HSCT group, we further defined two subgroups of women by evaluating the independent effects of the type of MAC on uterine volume: a group of women with hypoestrogenism composed of POI women non-treated with HRT versus a group having a “correct hormonal balance” composed of women treated with HRT for POI and women still having a persistent ovarian function with spontaneous periods.

Statistical analysis

Women baseline characteristics were reported descriptively. Categorical variables were summarized using counts and percentages and continuous variables using means and standard error of mean (SEM). Fischer’s exact and χ^2 tests were used to compare qualitative variables. Quantitative variables were compared using the student’s t-test. Uterine volume was compared according to MAC regimens and according to hormonal impregnation using Student’s t-test. The quartile distribution of age at HSCT was used to assess its potential impact on uterine volume. All statistical analysis was conducted using SPSS software. A p value $<.05$ was chosen for the statistical significance threshold.

Ethics

Written informed consent was obtained from each woman who participated in the FERTILEA study; The study was approved by the local Ethics Committee (N°IDRCB : 2017-A01596-47), registered at ClinicalTrials.gov/ NCT 03583294.

Results

Characteristics of the FERTILEA cohort

Thirteen L.E.A centers included 88 HSCT childhood AL long term survivor women in the FERTILEA study group. Mean age at HSCT was 9.1 ± 0.3 years with a mean follow-up duration of 16.4 ± 0.5 years. Mean age at evaluation was 26.5 ± 6.3 years in the HSCT group

Vs 25.6 ± 4.9 years in the control group. The FERTILEA population study characteristics are summarized in table 1. Among the 88 women included in HSCT group, two types of conditioning regimens were compared to the control group: alkylating agents-based regimen group (n=34) and TBI-based regimen group (n=54). In this last group, two MRI scans were not available. Among the 88 women included in HSCT group, 77 women were regarded as having a correct hormonal estroprogestative balance either by means of HRT in case of POI (n = 60) or in case of a persistent ovarian function with spontaneous menstruations (n = 17).

Primary endpoint

Table 2 reports mean uterine volumes revealed by MRI according to the type of MAC regimen received. In the control population, the volume of the uterus was 79.7 ± 3.3 mL. Uterine volume was significantly decreased in all HSCT survivor women ($p < 0.0001$). After alkylating agents-based regimen, the uterine volume was 45.3 ± 5.6 mL, corresponding to a significant volume reduction of 43.1 % [28.8 – 57.4] compared to the control group. After TBI, the uterine volume was 19.6 ± 1.9 mL, corresponding to a significant volume reduction of 75.3 % [70.5 – 80.2] compared to the control group.

Figure 1 shows uterine volume according to MAC regimen and hormonal status. In the alkylating agents-based regimen group, uterine volume was dramatically decreased in POI women without HRT compared to those who had a hormonal impregnation (15.2 ± 2.6 Vs 49.3 ± 6 mL, $p < 0.05$). However, despite a correct hormonal balance, uterine volume was still decreased when compared to control group. By contrast, after TBI, uterine volume was similar in all women, with no positive effect of hormonal impregnation on uterine volume (respectively 16.3 ± 2.6 Vs 20.1 ± 2.2 mL).

Figure 2 shows the effect of age at HSCT on uterine volume (using the quartile distribution) in the two conditioning regimen groups. In alkylating agents-based regimen group, uterine volume decreased significantly when HSCT was performed in 10 years old and

older girls. In the TBI group, uterine volume was significantly smaller if TBI was performed in childhood before 10 years of age.

Pubertal status at the time of transplantation was only known for 54 women. In the TBI group, uterine volume in adulthood was not different in women who were not menstruated at the time of HSCT ($n = 14$) vs women menstruated at the time of transplantation ($n = 20$) with respective uterine volumes of 18.8 ± 3.7 mL and 28.4 ± 2.3 mL (NS). In the alkylating agents-based regimen group, women already menstruated at the time of HSCT ($n = 10$) had a significantly decreased uterine volume compared to women not menstruated at the time of transplantation, with uterine volume of 26.6 ± 3.5 mL and 71.7 ± 13.1 mL respectively ($p = 0.007$).

In the alkylating agents-based regimen, we did not observe a correlation between CED and the uterine volume due to lack of data, as there were too few women exposed to alkylating agents at doses different from the average of $11\ 217$ mg/m² (supplemental figure 1).

Secondary outcomes

Uterine body-to-cervix ratio was significantly decreased both after alkylating agents-based regimen and after TBI, with respectively 1.3 ± 0.06 and 1.3 ± 0.04 Vs 1.7 ± 0.03 in control population ($p < 0.001$). ADC values of the myometrium was significantly decreased both after alkylating agents-based regimen and after TBI, with respectively 1.2 ± 0.05 and 1.3 ± 0.03 Vs $1.5 \pm 0.02 \times 10^{-3}$ mm²/s in control population ($p < 0.01$).

Pregnancy outcome in the HSCT survivor women

Among the 88 cases, 44 of them were living as a couple and 22 (25%) were planning to have a child. A total of 18 women (20.4 %) reported at least one pregnancy for a total of 8 live-births including 14 women who had at least one spontaneous pregnancy leading to 6 live-births. Only women from the alkylating agents-based regimen group obtained a live-birth

after spontaneous pregnancy. In the TBI regimen group, 3 women conceived after oocytes donation leading to one miscarriage and two live-births, and one woman had an early miscarriage after artificial insemination. Among the 14 women who reported a spontaneous pregnancy (15.9 % of the cohort), 4 reported an unplanned pregnancy that led to a medical abortion, 3 women had at least one child with a total of 6 live-births, 7 were childless after miscarriages. Among the spontaneous pregnancies, we observed a total of 6 early miscarriages and 11 late miscarriages. Thus, there were significantly more live births in the alkylating agents-based regimen group than in the TBI group: 60% Vs 11% ($p=0.03$). Too few pregnancies were reported to allow us to find a correlation between CED, uterine volume and pregnancy outcome.

Discussion

Until recently, only radiotherapy was known to induce damage on the uterus and to have an impact on obstetrical outcomes after cancer. Our study is the first to report by MRI in a homogeneous population of acute leukemia survivors a deleterious effect of HSCT after alkylating agents-based regimen on myometrium and uterine volume. Our results complement data from two previous studies by Beneventi *et al.* and van de Loo *et al.* who also reported uterine damage after high dose of chemotherapy, but their results were obtained by pelvic ultrasound scan imaging in a heterogeneous group of cancer survivor women (9,10).

In their study, Beneventi *et al.* compared 127 women, treated by HSCT during childhood, to 64 age-matched control women (9). Their population study included numerous types of malignant and nonmalignant hematologic diseases and solid tumors, leading to a heterogeneous population of post-HSCT survivors, exposed to various chemotherapy protocols. This study was the first to report a significant impact of MAC regimen using busulfan on adult uterine size: uterine volume was reduced by 81.9% (95 % CI, 71.8 – 87.8) after TBI and by 67.4 % (95 % CI, 58.5 – 75.6) after a busulfan conditioning regimen,

compared with women who had not received a MAC. However, they used two-dimensional ultrasound assessment of the uterus. To calculate uterine volume, they measured a straight line from the external orifice of the cervix to the uterine fundus, instead of measuring the anatomical length of the uterus which takes into account the angulation of the uterine body in relation to the cervix (i.e. including the measurement of the cervix length and then the uterine isthmus at the fundus). This measurement method may have underestimated the final calculated uterine volume.

Van de Loo et al. studied uterine volume by 3D transvaginal ultrasound technique in 55 childhood cancer survivors (CCSs) previously exposed to radiotherapy (abdominal-pelvic radiotherapy or TBI) and compared them with two female control groups : CCSs non-exposed to radiotherapy (n=110) and healthy women (n=110). They observed that uterine volume was smaller in the non-exposed to radiotherapy group of CCSs Vs healthy women (OR 2.61, 95% CI 1.15 – 5.91). However, the cases were heterogeneous with different types of cancers and various chemotherapy protocols and/or levels of radiotherapy dose (10).

An important question raised here is whether the decrease in uterine volume after high-dose chemotherapy is due to hormonal deprivation induced by ovarian damage, or to a specific “uterotoxic” impact of alkylating agents, or both? Meirrow et al. reported microvascular changes in ovarian stroma, with thickening and hyalinization of blood vessels with focal ovarian fibrosis in chemotherapy-exposed ovaries (11). In a similar manner, alkylating agents could have a substantial histological impact on myometrial tissue. However, our anatomical findings are not able to explain the etiology of the reduced uterine volume after chemotherapy. We can't exclude an impact of hormonal deprivation around puberty and adolescence. One limitation of our study is that we do not have accurate data on HRT compliance. Another hypothesis is that HRT doesn't provide the same hormonal impregnation of uterus compared to endogenous ovarian estrogens. In HRT, progestins and estrogens are

combined in numerous regimen (sequential cyclic continuous cyclic, continuous-combined, or cyclic combined), with different types, doses, routes of administration. In the FERTILEA cohort, the HRT protocols were individualized and were consequently too heterogeneous to study their specific impact on uterine volume.

However, in the alkylating agents-based regimen group, we observed that the uterine volume of women menstruated at the time of HSCT was significantly decreased compared to that of women not menstruated at the time of transplantation. The results of this sub-group analysis were surprising and provide an additional argument for an independent impact of alkylating agents on a post-pubertal uterus. Because of the number of missing data, these results should be treated with caution. Even though all women included in the study had their hormonal status assessed by a gynecologist at the time of inclusion, we could not rule out possible HRT compliance problems between the induction of puberty or between the POI and the initiation of HRT during the 16 years of follow-up.

We observed a significant decreased signal on ADC in the myometrium after HSCT, suggesting an architectural reorganization of the tissue and a possibly chemotherapy-induced fibrosis. ADC measurement has been described as a non-invasive tool able to diagnose cirrhosis in patients with chronic viral hepatitis (14). So, eventually, regardless of the physiopathology, our finding is that women after HSCT have a decreased uterine volume whatever the myeloablative conditioning treatment.

Regarding the impact of TBI, our results are in agreement with all the previous studies that reported that uterine volume is dramatically decreased in case of TBI before puberty, with no benefit of HRT on it (7).

Very surprising were our results showing the impact of the type of MAC regimen depending on age at HSCT. Because of the double-blinded measurement methodology by specialist radiologists, we believe that there is no significant bias in these results. The strength

of our study is to have included a homogeneous population of cases, all treated for the same pathology with similar MAC regimens. After TBI, uterine volume was strongly decreased when the irradiation took place early before puberty. The higher vulnerability of the prepubertal uterus exposed to uterine radiation has already been reported, due to early fibrosis induced by radiotherapy and alterations in blood supply (5,9,16). In contrast, in children and young adolescents treated with high dose of alkylating agents, the impact on uterine volume was highest when the treatment occurred post-puberty. We may assume that radiotherapy and chemotherapy use different mechanisms to damage the myometrium. Additionally, a post-pubertal exposure to high dose alkylating agents exposes adolescents to a higher risk of POI, inducing peripubertal estrogen deficiency that could also have a negative impact on final uterine volume.

POI and the subsequent hypoestrogenic state could be a confounding factor in women who had a poor compliance to HRT. Our results suggest that the impact of the MAC regimen on the uterus volume is not due to the POI-induced hypoestrogenism. Indeed, we observed a dramatic decrease in uterine volume in POI women without HRT, compared to women with POI and HRT and women still having spontaneous menstruations. However, in this last group, uterine volume was still significantly decreased compared to control group. In contrast to the TBI group, the uterine volume of women exposed to high dose of alkylating agents is less small in case of HRT or in women still having a persistent ovarian function. We lack data to explain why uterine volume would be less affected in case of chemotherapy exposure before puberty. Perhaps alkylating agents would be more cytotoxic on a pubertal growing uterus with differentiated myometrial cells. These results need to be confirmed by further studies.

The strengths of our study were to have had access to the large French LEA database, with a homogeneous population of HSCT survivors with standardized MAC regimens, and the

blinded MRI analysis in a single investigator center. The limitation of our study is that we haven't collected enough data to correlate age at HSCT, MAC regimen, uterine volume with the chance of spontaneous fertility and the obstetrical outcome.

Despite the possibility of spontaneous pregnancy, there is a high incidence of POI and infertility after HSCT. At present, there are two main strategies for infertile women who desire a pregnancy after HSCT: ovarian tissue transplantation (OTT) of tissue cryopreserved before HSCT (17–19) or oocyte donation for women who haven't had ovarian tissue cryopreservation before HSCT (20). The question of the impact of the HSCT on the uterus is therefore of major importance. The outcome of the pregnancy will depend in part on the volume and the vascularization of the uterus. When TBI occurs in childhood, the reduction of uterus volume seems irreversible despite a correct HRT. Even in adulthood, as underlined by Dolmans et al. who reported a live-birth rate of 19 % after OTT in a context of a history of pelvic irradiation (21), fibrosis of the uterus could explain increasing early and late miscarriage, preterm birth and small for gestational age (10). After TBI, we confirm data already described with a very low live-birth rate (11 %). Although the outcome of pregnancy was not the main objective of this study, we think it could be of interest. We observed a much lower live-birth rate (60 %) after high dose of chemotherapy, than what is reported in the general population (around 85%). Van de Loo *et al.* reported an increased risk of preterm delivery (<37 wk) in non-radiotherapy-exposed Childhood Cancer Survivors Vs control (OR = 4.21, 95 % CI 1.02-17.32). In a study that included only 8 POI women after chemotherapy, Sauer et al. reported a high miscarriage rate (57.4%) after oocyte donation in childhood cancer survivors (22). In contrast, in a study including 44 women in a program of oocyte donation after a history of chemotherapy, Munoz et al. didn't observe any difference concerning miscarriage rate and delivery rates of exposed Vs unexposed women (20). Data on pregnancy outcome after alkylating agents-based regimen are scarce because a lot of women

present POI after HSCT and are infertile. A large cohort study of pregnancy outcome after oocyte donation after high-dose chemotherapy would be highly interesting, but it would be very hard to conduct in the real life.

Conclusion

Our study adds supplementary data to the previous studies of Beneventi *et al.* and van de Loo *et al.* concerning a potential impact of chemotherapy on the myometrium and uterine volume (9,10). The impact of chemotherapy containing alkylating agents on myometrium and uterus needs to be further investigated. However, we also can assume a possible responsibility of a post-HSCT hormonal deprivation around puberty, or a poor HRT compliance, or a different impact of HRT on uterus compared to endogenous ovarian estrogens. Whatever the mechanisms of uterine volume reduction, we think that all pregnancies after HSCT should be managed as high risk of complications within a multidisciplinary care setting.

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Reference List

1. Diesch-Furlanetto T, Gabriel M, Zajac-Spychala O, Cattoni A, Hoebe BAW, Balduzzi A. Late Effects After Haematopoietic Stem Cell Transplantation in ALL, Long-Term Follow-Up and Transition: A Step Into Adult Life. *Front Pediatr* 2021;9:773895.
2. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. *Blood* 2014;124:344–53.
3. Gooptu M, Kim HT, Ho VT, Alyea EP, Koreth J, Armand P, et al. A Comparison of the Myeloablative Conditioning Regimen Fludarabine/Busulfan with Cyclophosphamide/Total Body Irradiation, for Allogeneic Stem Cell Transplantation in the Modern Era: A Cohort Analysis. *Biol Blood Marrow Transplant* 2018;24:1733–40.
4. van den Berg MH, van Dijk M, Byrne J, Berger C, Dirksen U, Winther JF, et al. Treatment-related fertility impairment in long-term female childhood, adolescent and young adult cancer survivors: investigating dose-effect relationships in a European case-control study (PanCareLIFE). *Hum Reprod* 2021;36:1561–73.
5. Holm K, Nysom K, Brocks V, Hertz H, Jacobsen N, Müller J. Ultrasound B-mode changes in the uterus and ovaries and Doppler changes in the uterus after total body irradiation and allogeneic bone marrow transplantation in childhood. *Bone Marrow Transplant* 1999;23:259–63.
6. Beneventi F, Locatelli E, Giorgiani G, Zecca M, Locatelli F, Cavagnoli C, et al. Gonadal and uterine function in female survivors treated by chemotherapy, radiotherapy, and/or bone marrow transplantation for childhood malignant and non-malignant diseases. *BJOG* 2014;121:856–65.

7. Rozen G, Rogers P, Chander S, Anderson R, McNally O, Umstad M, et al. Clinical summary guide: reproduction in women with previous abdominopelvic radiotherapy or total body irradiation. *Hum Reprod Open* 2020;2020:hoaa045.
8. Griffiths MJ, Winship AL, Hutt KJ. Do cancer therapies damage the uterus and compromise fertility? *Hum Reprod Update* 2020;26:161–73.
9. Beneventi F, Locatelli E, Giorgiani G, Zecca M, Mina T, Simonetta M, et al. Adolescent and adult uterine volume and uterine artery Doppler blood flow among subjects treated with bone marrow transplantation or chemotherapy in pediatric age: a case-control study. *Fertil Steril* 2015;103:455–61.
10. van de Loo LEXM, van den Berg MH, Overbeek A, van Dijk M, Damen L, Lambalk CB, et al. Uterine function, pregnancy complications, and pregnancy outcomes among female childhood cancer survivors. *Fertil Steril* 2019;111:372–80.
11. Meirow D, Dor J, Kaufman B, Shrim A, Rabinovici J, Schiff E, et al. Cortical fibrosis and blood-vessels damage in human ovaries exposed to chemotherapy. Potential mechanisms of ovarian injury. *Hum Reprod* 2007;22:1626–33.
12. Berbis J, Michel G, Baruchel A, Bertrand Y, Chastagner P, Demeocq F, et al. Cohort Profile: the French childhood cancer survivor study for leukaemia (LEA Cohort). *Int J Epidemiol* 2015;44:49–57.
13. Green DM, Nolan VG, Goodman PJ, Whitton JA, Srivastava D, Leisenring WM, et al. The cyclophosphamide equivalent dose as an approach for quantifying alkylating agent exposure: a report from the Childhood Cancer Survivor Study. *Pediatr Blood Cancer* 2014;61:53–67.
14. Charatcharoenwittaya P, Sukonrut K, Korpraphong P, Pongpaibul A, Saiviroonporn P. Diffusion-weighted magnetic resonance imaging for the assessment of liver fibrosis in chronic viral hepatitis. *PLoS One* 2021;16:e0248024.

15. Chen B, Xiao Z, Lv F, Lv F, Sheng B, Shi X. An analysis of apparent diffusion coefficient in the myometrium of normal uterus between the menopausal and premenopausal phases. *Jpn J Radiol* 2015;33:455–60.
16. Bath LE, Critchley HO, Chambers SE, Anderson RA, Kelnar CJ, Wallace WH. Ovarian and uterine characteristics after total body irradiation in childhood and adolescence: response to sex steroid replacement. *Br J Obstet Gynaecol* 1999;106:1265–72.
17. Donnez J, Dolmans M-M. Fertility Preservation in Women. *N Engl J Med* 2017;377(17):1657–65.
18. Shapira M, Raanani H, Barshack I, Amariglio N, Derech-Haim S, Marciano MN, et al. First delivery in a leukemia survivor after transplantation of cryopreserved ovarian tissue, evaluated for leukemia cells contamination. *Fertil Steril* 2018;109:48–53.
19. Sonmezer M, Ozkavukcu S, Sukur YE, Kankaya D, Arslan O. First pregnancy and live birth in Turkey following frozen-thawed ovarian tissue transplantation in a patient with acute lymphoblastic leukemia who underwent cord blood transplantation. *J Assist Reprod Genet* 2020;37:2033–43.
20. Muñoz E, Fernandez I, Martinez M, Tocino A, Portela S, Pellicer A, et al. Oocyte donation outcome after oncological treatment in cancer survivors. *Fertil Steril* 2015;103:205–13.
21. Dolmans M-M, von Wolff M, Poirot C, Diaz-Garcia C, Cacciottola L, Boissel N, et al. Transplantation of cryopreserved ovarian tissue in a series of 285 women: a review of five leading European centers. *Fertil Steril* 2021;115:1102–15.
22. Sauer MV, Paulson RJ, Ary BA, Lobo RA. Three hundred cycles of oocyte donation at the University of Southern California: assessing the effect of age and infertility diagnosis on pregnancy and implantation rates. *J Assist Reprod Genet* 1994;11:92–6.

Figure 1: Uterine volume according to the myeloablative conditioning (MAC) regimen and the hormonal status: POI women non-treated with hormone replacement therapy (HRT) Vs women having a “correct hormonal balance” composed by women treated with HRT for POI and women still having a persistent ovarian function with spontaneous periods.

Figure 2: Effect of age at hematopoietic stem cell transplantation (using a quartile distribution) on uterine volume by type of myeloablative conditioning (MAC) regimen.

Supplemental figure 1: Correlation between uterine volume and the mean calculated cumulative alkylating agents' dose per patient, using the cyclophosphamide equivalent dose (CED) in alkylating agents-based regimen group (n = 34).

Table 1: FERTILEA population study. Hematopoietic Stem Cell Transplantation (HSCT) childhood AL survivors' characteristics (n = 88). Values are presented as number (percentage), unless indicated otherwise. MAC = Myeloablative Conditioning; TBI = Total Body Irradiation

FERTILEA population study (n =88)	Total	Alkylating agents based regimen (n = 34)	TBI (n = 54)	p
Type of Leukemia				
Lymphoblastic acute leukemia	56 (64)	8 (24)	48 (89)	<.0001
Myeloid acute leukemia	32 (36)	26 (76)	6 (11)	
Type of HSCT				
Autograft	10 (11)	4 (12)	6 (11)	NS
Allograft	78 (89)	30 (88)	48 (89)	
Age at HSCT				
< 9 years	21 (43)	11 (32)	27 (50)	NS
> = 9 years	67 (57)	23 (68)	27 (50)	
MAC regimen before HSCT	88	34	54	
Ovarian tissue cryopreservation before HSCT	18 (21)	8 (25)	10 (19)	NS
Parenting plan at the time of evaluation	22 (28)	5 (16)	17 (36)	0.07
Age at time of the study (years ± SD)	26.5 ± 6.3	24.8 ± 1	27 ± 0.8	NS
Women regarded as having a correct estrogenic balance No (POI without HRT)				

Yes	11 (13)	4 (12)	7(13)	
	77 (88)	30 (88)	47 (87)	NS

Table 2: Comparison of MRI parameters of the uterus according to the type of myeloablative conditioning (MAC) regimen vs control group. TBI = Total Body Irradiation

ADC = apparent diffusion coefficient.

	Control group (n=88 ?)	Alkylating agents based regimen (n = 34)	TBI (n = 54)	Control vs. Alkylating agents	Control vs. TBI	Alkylating agents vs. TBI
Uterine volume (mL)	79.7 ± 3.3	45.3 ± 5.6	19.6 ± 1.9	< .0001	< .0001	< .001
Uterine body-to- cervix ratio	1.7 ± 0.03	1.3 ± 0.06	1.3 ± 0.04	< .0001	< .0001	NS
ADC (10 ⁻³ mm ² /s)	1.5 ± 0.02	1.2 ± 0.05	1.3 ± 0.03	< 0.001	< 0.001	NS



