ORIGINAL ARTICLE: FERTILITY PRESERVATION

Effect of first line cancer treatment on the ovarian reserve and follicular density in girls under the age of 18 years

Meryam El Issaoui, M.D.,[a] Veronica Giorgione, M.D.,[a] Linn S. Mamsen, M.Sc.,[a] Catherine Rechnitzer, M.D.,[b] Niels Birkebæk, M.D.,[c] Niels Clausen, M.D.,[c] Thomas W. Kelsey, Ph.D.,[d] and Claus Yding Andersen, D.M.Sc.[a]

[a] Laboratory of Reproductive Biology, Juliane Marie Centre for Women, Children and Reproduction, Copenhagen University Hospital and Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [b] Department of Paediatrics and Adolescent Medicine, Juliane Marie Centre, Copenhagen University Hospital, Copenhagen, Denmark; [c] Department of Pediatrics, Aarhus University Hospital, Aarhus, Denmark; and [d] School of Computer Science, University of St. Andrews, St. Andrews, United Kingdom

Objective: To study the impact of first-line antineoplastic treatment on ovarian reserve in young girls returning for ovarian tissue cryopreservation (OTC) in connection with a relapse.
Design: Retrospective case-control study.
Setting: University hospitals.
Patient(s): Sixty-three girls under the age of 18 years who underwent OTC before (group 1: 31 patients) and after (group 2: 32 patients) their initial cancer treatment.
Intervention(s): None.
Main Outcome Measure(s): Follicular densities (follicles/mm^3) measured from an ovarian cortical biopsy before OTC. The ovarian volume (mL) of entire ovaries excised for OTC was also monitored.
Result(s): There was no statistically significant difference in the mean age or follicular density between groups 1 and 2 (334 $\pm$ 476/mm^3 vs. 327 $\pm$ 756/mm^3). In contrast, the ovarian volume and total number of ovarian cortex chips cryopreserved were statistically significantly lower in patients who received gonadotoxic treatment before OTC (mean $\pm$ standard deviation [SD]: ovarian volume, 5.3 $\pm$ 3.1 mL vs. 2.9 $\pm$ 2.1 mL, respectively; number of cortex chips: 21.3 $\pm$ 8.1 vs. 15.2 $\pm$ 7.1, respectively). The reduction in the estimated ovarian reserve ranged from 10% to 20% in children to around 30% in adolescent girls (>10 years).
Conclusion(s): Girls under the age of 10 tolerate a gonadotoxic insult better than adolescents, who may experience up to a 30% reduction in the ovarian reserve via first-line gonadotoxic treatment, which at present is considered to have little effect on the follicle pool. This information will improve counseling of young female cancer patients in deciding whether to undergo fertility preservation treatment. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Fertility preservation, follicle density, ovarian tissue cryopreservation, ovarian volume

Discuss: You can discuss this article with its authors and with other ASRM members at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/11753-the-effect-of-first-line-cancer-treatment-on-the-ovarian-reserve-and-follicular-density-in-girls-under-the-age-of-18-years

During the last 50 years, cancer diagnoses in children and adolescents have progressed from being a serious potentially fatal disease to a most often curable disease. The 5-year survival for all cancer types is estimated to be more than 80% in both children and adolescents (1). Furthermore, the late-effect mortality from any cause has statistically significantly decreased across the last decades among 5-year survivors of childhood cancer according to the Childhood Cancer Survivor Study (2). This success comes from a remarkable development of effective therapeutic regimens, including alkylating agent-based chemotherapy and radiotherapy.

This progress has created an awareness of quality of life aspects after

Received May 4, 2016; revised August 24, 2016; accepted September 2, 2016.
M.E. has nothing to disclose. V.G. has nothing to disclose. L.S.M. has nothing to disclose. C.R. has nothing to disclose. N.B. has nothing to disclose. N.C. has nothing to disclose. T.W.K. has nothing to disclose. C.Y.A. has nothing to disclose.
Supported by the Child Cancer Foundation (grant 2012-26) in Denmark, the Novo Nordisk Foundation, and the EU Interregional Project ReproHigh/ReproUnion. They had no role in the study design, collection and analysis of data, data interpretation, or writing of the report.
Reprint requests: Claus Yding Andersen, D.M.Sc., Laboratory of Reproductive Biology, Section 5712, Juliane Marie Centre for Women, Children and Reproduction, University Hospital of Copenhagen, University of Copenhagen, Blegdamsvej 9, Rigshospitalet, DK-2100 Copenhagen, Denmark (E-mail: yding@rh.dk).

Fertility and Sterility® Vol. ■, No. ■, ■ 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.09.001

cancer, highlighting that successful treatment may compromise fertility after recovery (3). The pool of ovarian follicles that constitute the reproductive potential of a girl may be severely reduced or disappear as a consequence of the treatment required (4). In prepubertal girls the only available option to preserve fertility is to cryopreserve ovarian tissue (5, 6). If ovarian activity is destroyed and premature ovarian insufficiency (POI) occurs in young female survivors, cortical ovarian tissue might be transplanted to restore ovarian function for fertility purposes (7, 8). Recently, the first child born after transplantation of ovarian tissue that was harvested before menarche but after puberty was reported (9).

However, it is often difficult to decide whether it is necessary for a young girl with cancer to undergo an invasive procedure to obtain ovarian tissue for fertility preservation. The fertility preservation intervention is recommended when there is an estimated risk of POI exceeding 50% (10, 11). Ovaries from girls and young women contain a very high number of follicles and may tolerate a relatively high gonadotoxic insult without losing all follicles (12). In most cases treatment is initiated with low-risk regimens, but if more aggressive treatment is needed, harvesting ovarian tissue for fertility preservation will be considered. The question thus arises as to whether ovarian tissue cryopreservation (OTC) should be considered in connection with a first-line treatment such as ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine), which is often considered to cause a relatively mild gonadotoxic insult. Thus, the question is: What is the potential gonadotoxic insult caused by a first-line cancer treatment on the fertility potential in prepubertal and adolescent girls? To answer this question we evaluated the follicular density and ovarian volume in our cohort of young girls below the age of 18 years with respect to whether they had received gonadotoxic treatment before OTC.

MATERIALS AND METHODS

Patients

This retrospective study included a total of 63 girls younger than 18 years (range: 1.5–17.9 years) with a cancer diagnosis who had been referred to one of the three centers that participate in the Danish program for fertility preservation by OTC between the years 2002 and 2014. The number of patients who had not received chemotherapy before oophorectomy was 31 (group 1), and a total of 32 patients (group 2) had received low-risk gonadotoxic treatment before ovarian excision. All the patients in group 2 had been treated for an original oncologic diagnosis, had experienced a relapse, and had undergone OTC before further treatment. Patients were only included if a biopsy sample of their ovarian cortex was spared for histology in connection with OTC.

Procedure

The ovarian cortex was isolated by manual dissection and cut into pieces of approximately 5 × 5 mm and 1 mm thickness and frozen by slow-freezing technique as previously described elsewhere (13, 14). A small ovarian cortical biopsy (≈2 × 2 × 1 mm) is routinely taken for histologic examination before freezing. The piece was processed for histology, cut into 30-μm sections, and stained with periodic-acid Schiff reagents and Mayer hematoxylin. The follicular density, follicles per mm^3, was calculated by counting all types of follicles in every second section as previously described elsewhere (15). Because one entire ovary was removed, the ovarian volume was recorded by weighing the tissue before preparation for cryopreservation. The density of ovarian tissue has previously been determined to be 1 g/mL (16) using tissue weight and volume calculated by insertion in 0.9% NaCl solution. The ovarian surface area was calculated assuming that the ovarian volume represented a spherical structure.

Statistics

Microsoft Excel version 14.6.1 was used to analyze the data. The data for each variable for the pretreated and nonpretreated groups were symmetrically distributed with similar variances between groups 1 and 2, hence Student's *t*-test assuming equal variance was used to compare the between-group means of follicular density, ovarian volume, and number of ovarian cortex pieces (17). Age-adjusted comparisons were not performed because of the similar age characteristics (mean, median, interquartile range, range) between the two groups. $P<.05$ was considered statistically significant throughout the study. Quadratic regression curves were fitted to the data for both groups to visualize the similarities and differences reported (Figs. 1 and 2) and to estimate the age-related loss in ovarian reserve after treatment (Table 1).

The project of ovarian tissue cryopreservation was approved by the ethics committee of Copenhagen and Frederiksberg (H-2-2001-044). The storage and collection of patient data were approved by the Ministry of Health (J. no. 30-1372) and by the Danish authorities to comply with European Union tissue directives.

RESULTS

There was no statistically significant difference in mean age (±standard deviation [SD]) between groups 1 and 2 (13.2 ± 4.1 vs. 11.6 ± 4.3 years, $P=.19$. Cancer diagnoses for patients in the two groups are listed in Table 2. In group 1, the most frequent diagnoses were Hodgkin lymphoma (n = 8) and Ewing sarcoma (n = 6) whereas hematologic malignancies (acute lymphoblastic leukemia and acute myeloid leukemia, n = 14) were the most frequent in group 2. The chemotherapy regimens used in group 2 were all classified as having a low or moderate gonadotoxic impact as, for instance, low-dose alkylating agents. However, it has not been possible to recover information on the actual cancer treatments administered before excision of ovarian tissue. In all 63 patients a one-sided oophorectomy was performed to harvest ovarian tissue. No surgical complications were reported in connection with the oophorectomy.

Patients with leukemia received chemotherapy for relapse close to the OTC procedure (i.e., <1 month), while the patients with Ewing sarcoma/other sarcoma and other cancers received their last chemotherapy at months to years before OTC, depending on the time of relapse. However, these