Association of the minimal cyclophosphamide equivalent dose and outcome of microdissection testicular sperm extraction in patients with persistent azoospermia after chemotherapy

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Objective: To investigate whether the minimal cyclophosphamide equivalent dose (mCED), a novel approach for estimating alkylating agent exposure, is associated with the sperm retrieval rates by microdissection testicular sperm extraction (mTESE) in azoospermic post-chemotherapy cancer survivors.
Design: A retrospective cohort study conducted between 2002 and 2017.
Setting: An academic medical center.
Patients: A total of 28 azoospermic postchemotherapy cancer survivors who underwent mTESE.
Interventions: Chemotherapy exposure and mCED calculation.
Main Outcome Measures: The primary outcome was the association between the mCED and sperm retrieval rate using mTESE. The mCED value for each patient's regimen received was estimated using the lowest recommended dosing regimen from the range of recommended doses at the time of administration.
Results: Spermatozoa were successfully retrieved in 11 (39.3%) of the patients. Age at the time of receiving chemotherapy and mCED were significant factors associated with sperm retrieval. An mCED of $<4{,}000$ mg/m^2 had a higher sperm retrieval rate (10/14, 71.4%) than an mCED of $>4{,}000$ mg/m^2 (0/8, 0). The hormone levels were not significantly different when comparing patients with and without successful sperm retrieval. Seminoma, nonseminomatous germ cell tumor, and acute lymphoblastic leukemia had favorable sperm retrieval rates—100% (2/2), 66.7% (2/3), and 66.7% (2/3), respectively—although the numbers of patients in each group were small.
Conclusion: Among this cohort of patients with cancer who required chemotherapy regimens, successful sperm retrieval by mTESE was only noted among cancer survivors receiving an mCED of $<4{,}000$ mg/m^2. (Fertil Steril Rep® 2024;5:95–101. ©2023 by American Society for Reproductive Medicine.)
Key Words: Azoospermia, chemotherapy, alkylating agents, sperm retrieval

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The numbers of cancer cases diagnosed and cancer deaths in the United States have gradually decreased over the last two decades, in part because of the efficacy of early detection and advances of treatment options in the modern era (1). According to the Surveillance, Epidemiology, and End Results database, cancer diagnosed in both sexes of reproductive age accounts for approximately 8% of the total cancer incidence between 2011 and 2015 (2). Although men aged <45 years accounted for a minority of this cohort, they had a more favorable 5-year survival rate of up to 78% (3). In addition to oncologic outcomes, fertility is important to consider in young men, especially those who have desire for biologic paternity. Surgery, chemotherapy, radiotherapy, or some combinations of the aforementioned are often used to treat cancer but are not without side effects. The gonadal toxic effects of cancer in combination with chemotherapy and radiotherapy could have grave repercussions and leave patients with permanent or transient infertility (4, 5).

Exposure of genotoxic chemotherapeutic agents poses a substantial hazard to rapidly dividing cells. Cells with high mitotic activity, such as germ cells, are theoretically more susceptible to toxicity from chemotherapy. As exemplified by numerous animal studies (6), alkylating agents disrupt deoxyribonucleic acid replication and repair mechanisms by adding an alkyl group to the guanine base of deoxyribonucleic acid, resulting in a significantly increased risk of chromosomal aberrations in spermatozoa. Yet, this mutagenic effect is less pronounced in stem cell spermatogonia, presumably because of their low mitotic activity (6). Previous studies have demonstrated the deleterious effects of alkylating chemotherapeutic agents on spermatogenesis, although individual variation in sensitivity to chemotherapeutic agents exists (4). These individual differences make it challenging to predict the fertility status of the patient at the end of treatment. The cyclophosphamide equivalent dose (CED), an algorithm used to calculate the cumulative dosage of alkylating agents multiplied by their relative toxicity in relation to cyclophosphamide, is highly correlated with posttreatment semen parameters. The incidence of azoospermia significantly increases when a patient receives a CED of >4,000 mg/m^2 (7). Should azoospermia persist after chemotherapy, surgical testicular sperm retrieval using an operative microscope, termed "microdissection testicular sperm extraction" (mTESE), is the standard of care to acquire sperm for in vitro fertilization or cryopreservation (8).

Determining the CED can be a daunting task for andrologists but is of paramount importance in determining a patient's fertility potential. Often, a patient's chemotherapy regimen was administered years before elected fertility treatment and in a different geographic location, making it extremely challenging to determine their CED. To account for these challenges, we developed a novel method to determine a patient's alkylating agent exposure, termed "minimal cyclophosphamide equivalent dose" (mCED). This novel approach estimates a patient's minimum alkylating agent exposure and is designed to serve as a more readily obtainable prognostic factor for the sperm retrieval rate via mTESE in azoospermic postchemotherapy cancer survivors.

MATERIALS AND METHODS

Patient Selection

After reviewing 437 patients who underwent mTESE between January 2002 and October 2017, we identified 36 azoospermic patients with a history of cancer; of those, 28 received chemotherapy before mTESE for sperm retrieval by a single surgeon (R.E.B.). The diagnosis of azoospermia was made only if there was an absence of sperm in the semen sample as well as the centrifuged pellet under a high-powered microscope according to the World Health Organization guidelines (9). Demographic data, including testicular size, age at the time of chemotherapy, age at the time of mTESE, cancer diagnosis, radiation dosage, and serum hormone levels of follicle-stimulating hormone (FSH), luteinizing hormone, testosterone, prolactin, and estradiol, were collected. A total of 18 patients with testosterone levels of <300 ng/dL were treated with clomiphene citrate, human chorionic gonadotropin, or an aromatase inhibitor (anastrozole) for at least 3 months before surgery. Chemotherapy regimens were obtained in 22 of the 28 postchemotherapy cancer survivors. Nine (40.9%) of the remaining 22 patients did not have any alkylating agent exposure. All patient data were collected in accordance with the Northwestern Memorial Hospital Institutional Review Board–approved study protocol. This cohort study was conducted according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.

Microdissection Testicular Sperm Extraction

A 2.5-cm incision was made transversely on the hemiscrotum and carried down to through the dartos muscle layer. The spermatic cord and testis were delivered, followed by opening of the tunica vaginalis. An avascular region of tunica albuginea on the anterior surface of the mid-pole portion of the testis was visualized under a magnification of 6× using the surgical microscope. An equatorial incision was then made through the tunica albuginea along the circumference of the testis to expose the testicular parenchyma. The seminiferous tubules were systematically examined for the presence of dilated tubules (>300 μm) with a magnification of 20× to 24×. Next, a testicular tissue sample was sharply excised and processed to make a wet preparation slide. An experienced andrology technician and our team concurrently inspected each wet preparation slide in the operating room using a phase contrast microscope to identify active spermatogenesis in real time, until spermatozoa were found, as proposed by Schlegel (10).

CED Calculation

The cumulative dose and fertility risk assessment applying the CED is calculated using the equation described by Green et al. (7), involving alkylating agents such as cyclophosphamide, ifosfamide, procarbazine, chlorambucil, carmustine, lomustine, melphalan, Thio-TEPA, nitrogen mustard, and busulfan. Chemotherapy combination treatments for these patients are as follows: ABVD (Adriamycin, bleomycin, vinblastine, and dacarbazine); MOPP (Mustargen, Oncovin, procarbazine, and prednisone); MOPP/ABVD; C-MOPP (cyclophosphamide, Mustargen, Oncovin, procarbazine, and prednisone); OPEC

TABLE 1

The minimal cyclophosphamide equivalent dose calculation.

Chemotherapy drug	Relative toxicity
Cyclophosphamide	1.0
Ifosfamide	0.244
Procarbazine	0.857
Chlorambucil	14.286
Carmustine (BCNU)	15.0
Lomustine (CCNU)	16.0
Melphalan	40.0
Thio-TEPA	50.0
Nitrogen mustard	100.0
Busulfan	8.823

Note: The calculation of the minimal cyclophosphamide equivalent dose is identical to that of the cyclophosphamide equivalent dose, employing the following equation: cyclophosphamide equivalent dose (mg/m^2) = 1.0 (cumulative cyclophosphamide dose [mg/m^2]) + 0.244 (cumulative ifosfamide dose [mg/m^2]) + 0.857 (cumulative procarbazine dose [mg/m^2]) + 14.286 (cumulative chlorambucil dose [mg/m^2]) + 15.0 (cumulative carmustine dose [mg/m^2]) + 16.0 (cumulative lomustine dose [mg/m^2]) + 40 (cumulative melphalan dose [mg/m^2]) + 50 (cumulative Thio-TEPA dose [mg/m^2]) + 100 (cumulative nitrogen mustard dose [mg/m^2]) + 8.823 (cumulative busulfan dose [mg/m^2]). The minimal cyclophosphamide equivalent dose value for each patient's administered regimen was estimated using the lowest recommended dosing regimen within the range of doses recommended at the time of administration.

Huang. mCED and sperm extraction outcome. Fertil Steril Rep 2024.

TABLE 2

Demographic data of azoospermic patients after chemotherapeutic treatment.

Characteristic	Study participants
Age at diagnosis (y)	
0–13	11
14–20	6
>20	11
Age at diagnosis (y)	
Median (range)	16 (2–38)
Mean ± SD	17.5 ± 10.1
Age at receiving mTESE	
Median (range)	35 (27–46)
Mean ± SD	34.3 ± 4.5
Elapsed time from chemotherapy to mTESE (y)	
Median (range)	20 (1–33)
Mean ± SD	16.8 ± 9.8
Pretreatment hormone level	
FSH (mean ± SD) (IU/L)	22.1 ± 15.0
LH (mean ± SD) (IU/L)	7.0 ± 3.9
Testosterone (mean ± SD) (ng/dL)	248.5 ± 131.6
Prolactin (mean ± SD) (ng/mL)	27.3 ± 11.5
Estradiol (mean ± SD) (pg/mL)	11.5 ± 5.4
Testicular size (mean ± SD) (mL)	12.5 ± 5.4

Note: FSH = follicle-stimulating hormone; LH = luteinizing hormone; mTESE = microdissection testicular sperm extraction; NSGCT = nonseminomatous germ cell tumor.

Huang. mCED and sperm extraction outcome. Fertil Steril Rep 2024.

(vincristine, cisplatin, etoposide, and cyclophosphamide); CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone); CVP (cyclophosphamide, vincristine, and prednisolone); and CALGB 8811 regimen (cyclophosphamide, etoposide, VP-16, vincristine, L-asparginase, methotrexate, and cytarabine). For the purposes of this study, the mCED value for each patient's regimen received was estimated using the lowest recommended dosing regimen from the range of recommended doses at the time of administration (11–16). Given that temozolomide and dacarbazine belong to the alkylating agent family of chemotherapy agents but are not included in the equation of the CED, the dosage of temozolomide or dacarbazine received by patients with cancer was not calculated in the mCED. The calculation method for determining the mCED, which relies on assessing the relative toxicity of individual chemotherapy agents, is comprehensively detailed in Table 1.

Statistical Analysis

Data were analyzed using SPSS Software V.20 (SPSS Inc., Chicago, IL). Statistical analyses were performed using Student's *t* test or the Mann-Whitney *U* test with the Shapiro-Wilk normality test. Interactions with categorical variables were examined using Fisher's exact test. The results were expressed as means ± SDs. Multiple logistic regression analyses were performed to assess the association of the mCED with the success of sperm retrieval, adjusting for potential confounding variables, including age at completing chemotherapy, FSH, testosterone, and testis size. The statistically significant value was set at $P<.05$ (P values are 2-tailed).

RESULTS

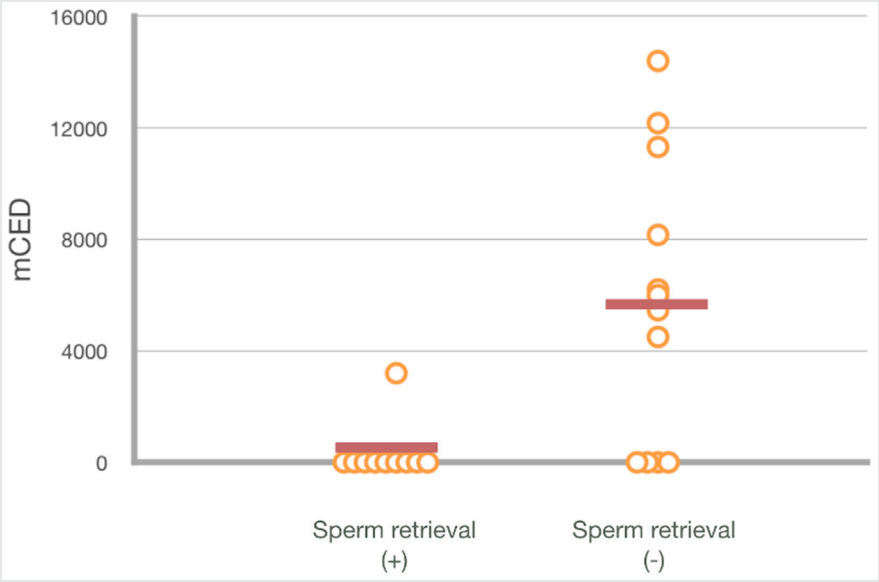
Demographic Data

A total of 28 patients, including 22 with the known chemotherapeutic regimen and 6 with an unknown history of alkylating agent exposure during chemotherapy treatment, were enrolled in the present series. The mean age at the time of receiving chemotherapy was 17.5 ± 10.1 years, and the mean age at the time of undergoing mTESE was 34.3 ± 4.5 years. Specific cancer diagnoses included non-Hodgkin lymphoma (n = 5), Hodgkin lymphoma (n = 4), acute lymphoblastic leukemia (ALL, n = 3), chronic myeloid leukemia (n = 2), testicular seminoma (n = 3), testicular nonseminomatous germ cell tumor (NSGCT, n = 3), rhabdomyosarcoma (n = 3), and other pathologies (one case in each of the following diagnosis: osteosarcoma; glioblastoma; neuroblastoma; neuroblastoma; mediastinal seminoma; primitive neuroendocrine tumor; and gliosarcoma). Table 2 summarizes the baseline characteristics of azoospermic patients after chemotherapeutic treatment. Prechemotherapy semen analysis data were available in two patients diagnosed with testicular NSGCT and gliosarcoma, and both of their results showed azoospermia.

Clinical Outcomes

Spermatozoa were successfully recovered in 11 (39.3%)of the 28 patients. The age at the time of receiving chemotherapy and mCED were associated with sperm retrieval ($P<.05$). For patients with an mCED of 0, the sperm retrieval rate was 69.2% (9/13). Patients with an mCED of <4,000 mg/m^2 had a higher sperm retrieval rate (10/14, 71.4%) than those with an mCED of >4,000 mg/m^2 (0; Fig. 1). Only one patient, who received alkylating agents (CALGB 8811 regimen), a patient with ALL and an mCED of 3,200 mg/m^2, had successful sperm retrieval.

FIGURE 1



Relation between the minimal cyclophosphamide equivalent dose and sperm retrieval outcome using microdissection testicular sperm extraction (means indicated by horizontal lines with each group).

Huang. mCED and sperm extraction outcome. Fertil Steril Rep 2024.

The spermatozoa retrieval rates were >50% in patients with ALL (2/3, 66.7%), chronic myeloid leukemia (1/2, 50%), Hodgkin lymphoma (2/4, 50%), testicular seminoma (2/2, 100%), testicular NSGCT (2/3, 66.7%), osteosarcoma (1/1, 100%), and glioblastoma (1/1, 100%). The testicular size was also significantly larger in patients with positive sperm recovery (15 ± 5.2 vs. 9.9 ± 4.5 mL, $P<.05$). Men with successful sperm retrieval had higher baseline testosterone levels than those with an unsuccessful sperm retrieval (294.9 ± 121.7 vs. 218.4 ± 132.3 mg/dL, respectively; $P<.05$). Other hormone parameters, FSH, luteinizing hormone, prolactin, and estradiol, did not show a significant difference between patients with and without successful sperm retrieval (Table 3). Four patients had treatment with alkylating agents not included in the CED equation: temozolomide in a patient with glioblastoma and in a patient with gliosarcoma and dacarbazine in two patients with Hodgkin lymphoma. Sperm were recovered in the patient with glioblastoma and two patients with Hodgkin lymphoma but not in the patient with gliosarcoma. The mTESE success rates were higher in men without alkylating exposure than those with alkylating exposure; however, this did not reach statistical significance (6/9, 66.7%, vs. 4/13, 30.8%; $P=.192$). Radiation exposure did not predict sperm recovery when administered to the hypothalamic/pituitary axis ($P=.62$) or pelvis ($P=.12$) (Table 3). Multiple logistic regression analysis (adjusted for confounders) for successful sperm retrieval showed that the chance of recovering sperm during mTESE cannot be predicted by any individual variable. The histologic testicular subtypes among patients with unsuccessful mTESE included "Sertoli cell only" in 14 patients, "Leydig cell hyperplasia" in 2 patients, and "hyalinized tissue" in 1 patient.

DISCUSSION

The present study demonstrates a close association between the mCED and successful sperm retrieval by mTESE. Compared with the current model, CED, the new mCED approach takes less information to calculate, which is relevant given the limited historical medical information that reproductive health specialists typically have access to when caring for cancer survivors. Given the mCED's relative ease of use, it should aid andrologists in more accurately providing patients with prognostic information when they present for mTESE, typically years after receiving cancer therapy.

The American Cancer Society recently estimated that there will be 1,806,590 new cancer diagnoses annually in the United States, including >50,000 cases in men aged <49 years (1). With survivorship in mind, fertility preservation is important to this young cohort. Nearly two decades ago, most oncologists discussed fertility issues with patients with cancer; however, only half of oncologists offered sperm cryopreservation, and even fewer patients (30%) actually banked their sperm before treatment (17). Within the past 20 years, oncologists' understanding of the cytotoxic effects of chemotherapy and its potential irreversibility on a patient's fecundity has become of paramount importance. In light of the underutilization of sperm cryopreservation by male patients with cancer, especially men of reproductive age, the American Society of Clinical Oncology published

TABLE 3

Patient characteristics stratified by sperm retrieval.

Characteristic	Sperm retrieval (+)	Sperm retrieval (−)	*P* value
Age at completing chemotherapy (y)	22.9 ± 7.5	14.0 ± 5.1	<.05
Age at receiving mTESE (y)	34.4 ± 5.1	34.3 ± 4.2	.98
Time from chemotherapy to mTESE (y)	11.5 ± 9.2	20.3 ± 8.8	<.05
FSH (IU/L)	15.2 ± 9.8	26.5 ± 16.4	.05
LH (IU/L)	5.8 ± 2.0	7.9 ± 4.6	.15
Testosterone (ng/dL)	294.9 ± 121.7	218.4 ± 132.3	<.05
Prolactin (ng/mL)	9.4 ± 3.7	7.6 ± 2.4	.27
Estradiol (pg/mL)	24.5 ± 13.4	29.1 ± 10.4	.32
Radiation treatment			
Hypothalamic/pituitary	1/11 (9.1%)	4/17 (23.5%)	.62
Pelvic region	0/11 (0%)	5/17 (29.4%)	.12
Alkylating agent exposure[a]	4/10 (40%)	9/12 (66.7%)	.192
mCED (mg/m^2)[a]	320 ± 1,011.9	5,683.2 ± 5,106.7	<.05
0	9/10 (90%)	4/12 (33.3%)	<.05
0–4,000	1/10 (10%)	0/12 (0%)	.45
4,000–8,000	0/10 (10%)	4/12 (33.3%)	.10
>8,000	0/10 (10%)	4/12 (33.3%)	.10
Testicular size (mL)	15 ± 5.2	9.9 ± 4.5	<.05

Note: Data presented as mean ± standard deviation, unless specified otherwise. FSH = follicle-stimulating hormone; LH = luteinizing hormone; mCED = minimal cyclophosphamide equivalent dose; mTESE = microdissection testicular sperm extraction.
[a] Data were available in 22 patients.

Huang. mCED and sperm extraction outcome. Fertil Steril Rep 2024.

recommendations about fertility preservation in patients with cancer in 2006 and updated them in 2013. These recommendations called for the clinician to "discuss at the earliest possible time the risk of fertility impairment" and "the prompt referral to a qualified fertility preservation specialist if the patient is interested" (18, 19). The objective for clinicians is to offer cryopreservation before cancer treatment (surgery, chemotherapy, and radiation) given its association with azoospermia.

Contemporary studies have reported that certain types of malignancies can have a detrimental effect on semen quality and that normal semen parameters for cryopreservation can only be expected in roughly 40% of patients (20). Even more severe testicular failure, the absence of sperm in the ejaculate, is not uncommon. The overall azoospermic rate at the time of cancer diagnosis is 11.6%, ranging from 3.9% to 15.3%, depending on the type of cancer (21). Surgical sperm retrieval remains the gold standard to obtain testicular sperm before cancer treatment in azoospermic men desiring fertility preservation. This technique dubbed "Onco-TESE" (oncologic testicular sperm extraction) first described by Schrader et al. (22) in 2003 reported successful sperm retrieval in 42.9% of patients with testicular cancer and 47.1% of those with malignant lymphoma. In a retrospective analysis of 73 postchemotherapy azoospermic men undergoing mTESE, the largest cohort to date, Hsiao et al. (8) demonstrated the highest sperm retrieval rate in patients with testicular cancer (84.6%), whereas those with sarcoma yielded the lowest sperm recovery rate (14.3%). However, hormone parameters were not predictive of successful spermatozoa retrieval through mTESE (8).

Traditional alkylating agent exposure metrics, such as the CED and alkylating agent dose, help clinicians determine the likelihood a patient will be azoospermic. However, adoption of CED allows the physician to quantify cumulatively the alkylating agent dosage rather than obtaining an "alkylator score" using the alkylating agent dose, which only represents an assessment of relative drug toxicity (23). Our objective was to identify predictors for successful sperm recovery in azoospermic posttreatment cancer survivors. Similar to the study by Hsiao et al. (8), alkylating agent exposure fails to predict successful sperm recovery completely by mTESE. Nevertheless, using a CED cutoff point of 4,000 mg/m^2 helps to predict the outcome of mTESE. Of note, none of our eight patients with an estimated mCED of >4,000 mg/m^2 had successful mTESE. On the contrary, men with an mCED of <4,000 mg/m^2 had viable sperm recovered in 10 of 14 cases. These results demonstrate similar findings as the emerging published body of literature regarding the association between the CED and semen parameter. To date, there is no safe CED value below which there is no risk of potentially irreversible fertility alterations (7).

Our study, similar to the study by Green et al. (7), indicates that high-dose alkylating agents (measured by the CED) cause hazardous disturbance in spermatogenesis. This suggests that a high CED gonadotoxic regimen not only causes genotoxic damage to the spermatocyte, spermatids, and spermatozoa but also leads to complete depletion of spermatogonial stem cells. With a CED of >4,000 mg/m^2, this damage predicts azoospermia in the ejaculate and a 100% failure rate of mTESE, resulting in permanent sterility.

Age at completing chemotherapy differed in patients with and without successful sperm retrieval, likely because of underlying cancer and treatment, rather than age. Among cancer survivors, those with testicular cancer are typically diagnosed at an older age and tend to have a higher

probability of sperm retrieval success. This is shown in our cohort that four of our five testicular cancer survivors with successful sperm retrieval had a mean age of 27.8 years when completing chemotherapy. Additionally, prepubescent patients who received chemotherapy are more at risk of reduced tubular fertility index (percentage of seminiferous tubules containing germ cells) and less likely to have spermatogenic recovery (24).

Our findings both corroborate and diverge from those reported by Brant et al. (25). In line with the investigation by Brant et al. (25), our investigation revealed that successful sperm retrieval was not achieved among patients who received chemotherapy accompanied by pelvic radiation, as indicated in Table 3. This observation suggests a potential adverse effect of pelvic radiation on sperm retrieval after chemotherapy. However, it is noteworthy that although radiation exposure did not emerge as a predictive factor for sperm recovery on administration to the pelvis ($P=.12$) in our study, this finding contrasts with the conclusions drawn by Brant et al. (25). Their study reported a diminished sperm retrieval rate among individuals subjected to pelvic radiation, compared with those who underwent chemotherapy exclusively or in combination with extrapelvic radiation. This discrepancy may be attributed to the relatively modest size of our study population, which could have constrained our ability to detect statistically significant differences in the sperm retrieval rates between the two groups.

Despite the promising results, our study should be viewed within its limitations. First, our study is retrospective. Second, we analyzed a relatively small sample size. Third, the cohort of men who retained sperm in the ejaculate after chemotherapy was not included in our study, thereby constraining the comprehensive application of the mCED as a predictive tool for assessing the fertility potential of all men treated with chemotherapy. Finally, exact alkylating agent exposure (measured by the traditional CED) was not available for review, which means that additional studies in a cohort with information of exact alkylating agent exposure are needed to examine the relation between the CED and sperm retrieval rate, because the mCED only represents a patient's minimum alkylating agent exposure. Despite these limitations, our study demonstrates the clinical utility of the mCED allowing physicians to better understand the chances of successful mTESE after chemotherapy. In general, a history of receiving any alkylating agents was associated with a low likelihood of successful sperm retrieval, and in our cohort, if the mCED was $>4{,}000$ mg/m^2, no patients had successful sperm retrieval.

CONCLUSION

Limited evidence exists for counseling postchemotherapy patients with azoospermia. Our study demonstrated that using the mCED as an adjunct, patients with an mCED of $<4{,}000$ mg/m^2 were found to have a significantly higher chance of a successful sperm retrieval than those with an mCED of $>4{,}000$ mg/m^2. These findings allow providers to determine which patients are ideal mTESE candidates and more accurately predict the chances of successful sperm retrieval.

Declaration of Interests

I-S.H. has nothing to disclose. R.J.F. has nothing to disclose. J.A.H. has nothing to disclose. J.W. has nothing to disclose. N.E.B. has nothing to disclose. M.N.P. has nothing to disclose. A.S. has nothing to disclose. W.J.H. has nothing to disclose. R.E.B. has nothing to disclose.

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