VOLUME 31 · NUMBER 10 · APRIL 1 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Lack of Specificity of Plasma Concentrations of Inhibin B and Follicle-Stimulating Hormone for Identification of Azoospermic Survivors of Childhood Cancer: A Report From the St Jude Lifetime Cohort Study

Daniel M. Green, Liang Zhu, Nan Zhang, Charles A. Sklar, Raymond W. Ke, William H. Kutteh, James L. Klosky, Sheri L. Spunt, Monika L. Metzger, Fariba Navid, DeoKumar Srivastava, Leslie L. Robison, and Melissa M. Hudson

Daniel M. Green, Liang Zhu, Nan Zhang, James L. Klosky, Monika L. Metzger, Fariba Navid, DeoKumar Srivastava, Leslie L. Robison, and Melissa M. Hudson, St. Jude Children's Research Hospital; Raymond W. Ke and William H. Kutteh, Fertility Associates of Memphis; Sheri L. Spunt, Monika L. Metzger, Fariba Navid, and Melissa M. Hudson, University of Tennessee Health Sciences Center, Memphis, TN; and Charles A. Sklar, Memorial Sloan-Kettering Cancer Center, New York, NY.

Published online ahead of print at www.jco.org on February 19, 2013.

Supported in part by Grant No. CA-21765 from the United States Public Health Service and by funding from the American Lebanese Syrian Associated Charities (to St. Jude Children's Research Hospital).

Presented in part at the 43rd Annual Conference of the International Society of Paediatric Oncology, Auckland, New Zealand, October 28-30, 2011.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Daniel M. Green, MD, Department of Epidemiology and Cancer Control, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Mail Stop 735, Memphis, TN 38105-2794; e-mail: daniel.green@stjude.org.

0732-183X/13/3110-1324/$20.00

DOI: 10.1200/JCO.2012.43.7038

ABSTRACT

Purpose
Many male survivors of childhood cancer are at risk for azoospermia. Although both the levels of follicle-stimulating hormone (FSH) and inhibin B are correlated with sperm concentration, their ability to predict azoospermia in survivors of childhood cancer remains uncertain.

Patients and Methods
Semen analysis was performed and serum levels of FSH and inhibin B were measured in 275 adult male survivors of childhood cancer who had received gonadotoxic therapy. Receiver operating characteristic (ROC) analysis was performed to determine the optimal inhibin B and FSH values for identifying patients with azoospermia. The patient sample was divided into a learning set and a validation set. Sensitivity, specificity, and positive and negative predictive value were calculated.

Results
Inhibin B was dichotomized as ≤ 31 ng/L or more than 31 ng/L and FSH was dichotomized as ≤ 11.5 mIU/mL or more than 11.5 mIU/mL based on results of the ROC analysis. Using these values, the specificity of the serum level of inhibin B for identifying azoospermic survivors was 45.0%, and the positive predictive value was 52.1%. The specificity for FSH was 74.1%, and the positive predictive value was 65.1%.

Conclusion
Neither serum inhibin B nor FSH is a suitable surrogate for determination of sperm concentration in a semen sample. Young men and their physicians should be aware of the limitations of these measures for assessment of fertility potential.

J Clin Oncol 31:1324-1328. © 2013 by American Society of Clinical Oncology

INTRODUCTION

The treatment of children and adolescents with cancer has become increasingly successful. Approximately 80% of all patients diagnosed before 15 years of age will survive for five years. The majority are expected to survive for many years after diagnosis.[1] In boys and young men, irradiation of the gonads and/or the use of certain classes of chemotherapeutic agents (eg, alkylating agents) may damage spermatogenesis leading to impaired fertility.[2]

Adult survivors of childhood cancer place high value on fertility and desire information regarding their potential to sire/conceive a pregnancy.[3,4] Semen analysis is a noninvasive method for estimation of male fertility potential, but religious, cultural, and personal barriers may deter individuals from completing the procedure.[5]

Inhibin B is a dimeric protein that consists of alpha and beta subunits that are synthesized in the Sertoli cells of the testis.[6] The level of inhibin B is inversely related to the level of follicle-stimulating hormone (FSH) in normal males and those with a variety of reproductive abnormalities.[7] Jensen et al[8] reported that the serum level of inhibin B was directly related, whereas the serum level of FSH was inversely related, to sperm concentration in healthy Danish males. Others have reported that the serum inhibin B level is highly correlated with sperm concentration among men undergoing evaluation for male factor infertility.[9-11] Studies of the relationship between serum levels of inhibin B and FSH and

sperm concentration in adult survivors of childhood cancer have been limited in the number of evaluated patients who received gonadotoxic treatment and/or the number of patients from whom semen samples were obtained.[12-14]

We undertook these analyses to determine the sensitivity, specificity, and positive and negative predictive value of the serum levels of inhibin B and FSH for the identification of azoospermia. This assessment is possible because of the availability of a large population of long-term survivors of childhood cancer who had been treated with alkylating agents, direct gonadal irradiation (any dose), and/or hypothalamic/pituitary irradiation (≥ 40 Gy), all of which are associated with a significant risk of azoospermia.[15]

PATIENTS AND METHODS

A cohort of patients (St. Jude Lifetime Cohort Study [SJLIFE]) was identified that fulfilled the following criteria: diagnosis of childhood malignancy treated at St. Jude Children's Research Hospital (SJCRH), survival ≥ 10 years from diagnosis, and current age ≥ 18 years. The detailed methods used for ascertainment, recruitment, and evaluation of the members of this cohort have been reported previously.[16] This investigation was approved by the institutional review board at SJCRH, and all participants and/or their legal guardians provided informed consent.

The cumulative doses for 32 specific chemotherapeutic agents (5-azacytidine, bleomycin, busulfan, carboplatin, carmustine, cisplatinum, cyclophosphamide [intravenously [IV] or orally], cytarabine [IV, intramuscularly, intrathecally, subcutaneously], dacarbazine, dactinomycin, daunorubicin, dexamethasone, doxorubicin, etoposide [IV, orally], fludarabine, fluorouracil, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, lomustine, melphalan, methotrexate [IV, intramuscularly, intrathecally], nitrogen mustard, prednisone, procarbazine, teniposide, thioguanine, thiotepa, tretinoin, vinblastine, vincristine), surgical procedures, and radiation treatment fields, dose, and energy source were abstracted from the medical records according to a protocol similar to that used in the Childhood Cancer Survivor Study (CCSS).[17]

Participants underwent a risk-based assessment as suggested by the Children's Oncology Group Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent and Young Adult Cancer (COG Guidelines).[18] Semen analysis was offered to men who had received potentially gonadotoxic treatment, including exposure to an alkylating agent, direct testicular irradiation (any dose), or hypothalamic/pituitary irradiation (≥ 40 Gy). FSH was to be measured in all men who received gonadotoxic therapy, and inhibin B was to be determined in all men who underwent semen analysis. Those who had received hypothalamic/pituitary irradiation ≥ 40 Gy or had a tumor in the hypothalamic/pituitary region (eg, craniopharyngioma) and those who were receiving exogenous androgen treatment were excluded from these analyses. Eleven participants had undergone unilateral orchiectomy. No participant underwent bilateral orchiectomy.

Inhibin B and FSH Analyses

To analyze the serum hormone levels, we obtained morning peripheral blood samples and separated the serum. The serum samples were stored in liquid nitrogen at −180° Celsius until they were shipped on dry ice to Quest Diagnostics (Valencia, CA) for inhibin B analysis by immunoassay. The lower limit of the assay is 30 ng/L.

Serum FSH was assayed by using a two-step sandwich-type electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN) in the SJCRH clinical laboratory. Within-assay coefficient of variation (CV) was 1.2% (n = 20; mean, 4.72 mIU/mL). Day-to-day imprecision for three control materials (Liquichek Immunoassay Plus Control, Trilevel; Bio-Rad Laboratories, Hercules, CA) was as follows: Level 1, n = 155; mean, 7.33 mIU/mL; CV, 3.9%; Level 2, n = 155; mean, 19.72 mIU/mL; CV, 3.7%; Level 3, n = 150; mean, 46.65 mIU/mL; CV, 3.7%. The normal range for males age 20 to 50 years in the SJCRH laboratory is 2.0 to 9.2 mIU/mL.

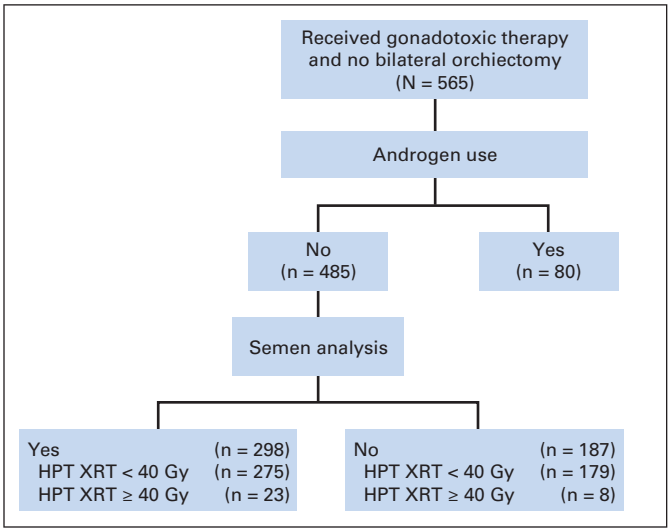

Fig 1. CONSORT diagram showing patient population from which semen analysis data were derived. HPT XRT, hypothalamic-pituitary irradiation.

Semen Analysis

The semen samples were collected after a minimum of 2 days and a maximum of 7 days of sexual abstinence and were processed within 30 minutes of collection following the WHO Guidelines, 5th Edition.[19] Every semen sample was allowed to liquefy, and the time to liquefaction was recorded. The raw sample was microscopically evaluated. If no sperm were detected, the sample was centrifuged and concentrated. The concentrated sample was again evaluated before it was considered azoospermic. A recent medical history was taken at the time of collection and, if any historical issues were revealed (recent fever above 102°F, certain medication use, recent genitourinary tract infection, or injury), a request for a repeat specimen in 1 month to confirm azoospermia was made.

Statistical Analysis

Receiver operating characteristic (ROC) analysis was undertaken to identify the optimal dichotomization values for FSH, inhibin B, and the inhibin B:FSH ratio for identifying patients with azoospermia. Specifically, the patient sample was divided into a learning set (n = 140) and a validation set (n = 135) by random assignment. The ROC analysis was performed by using PROC LOGISTIC in SAS 9.2 (SAS Institute, Cary, NC).

RESULTS

Study Population

Two hundred ninety-eight (53%) of 565 SJLIFE males who received gonadotoxic treatment, were not receiving exogenous androgens, and participated in SJLIFE before February 23, 2011, submitted a semen specimen for evaluation (Fig 1). Of these 298, we excluded those who had received ≥ 40 Gy hypothalamic/pituitary irradiation and/or had a tumor in the hypothalamic/pituitary region (n = 23), for a total of 275 eligible cases. The majority were non-Hispanic white and had at least a high school education. The most frequent diagnosis was acute lymphoblastic leukemia (Table 1). The median age at semen analysis was 30.5 years (range, 19.7 to 59.1 years). Inhibin B was measured in 238 and FSH in 275 participants. One hundred five (38.2%) of 275 were found to be azoospermic. Most of those who submitted semen specimens reported that they had no children (Appendix Table A1, online only). Among those who did not submit semen specimens, live births were reported by one participant who did

Table 1. Demographic Characteristics for Study Patients

Characteristic	No. of Patients	%
Race/ethnicity		
Non-Hispanic white	242	88.0
Non-Hispanic black	28	10.2
Other	5	1.8
Education		
No high school or GED	17	6.2
High school or GED	67	24.4
Some college; no bachelor's degree	96	34.9
Bachelor's degree or higher	89	32.4
Missing	6	2.1
Age at diagnosis, years		
> 0- < 5	109	39.6
≥ 5- < 10	72	26.2
≥ 10- < 15	61	22.2
≥ 15- < 20	32	11.6
≥ 20	1	0.4
Age at semen analysis, years		
≥ 18- < 26	74	26.9
≥ 26- < 36	127	46.2
≥ 36- < 56	73	26.5
≥ 56	1	0.4
Diagnosis		
Acute lymphoblastic leukemia	138	50.8
Acute myelogenous leukemia	4	1.5
CNS	16	5.8
Ewing sarcoma family of tumors	23	8.4
Hodgkin lymphoma	25	9.1
Non-Hodgkin lymphoma	15	5.5
Neuroblastoma	14	5.1
Osteosarcoma	17	6.2
Retinoblastoma	8	2.9
Rhabdomyosarcoma	7	2.6
Soft tissue sarcoma	2	0.7
Wilms tumor	5	1.8
Other	1	0.4

Abbreviation: GED, General Educational Development [test].

not complete the SJLIFE clinical evaluation, two who declined semen analysis, one who was unable to produce a semen specimen, one who had erectile dysfunction, and two who had undergone vasectomy.

Sensitivity, Specificity, and Positive and Negative Predictive Values

ROC analysis was performed to identify the optimum values of inhibin B, FSH, and the inhibin B:FSH ratio for identification of patients with azoospermia. On the basis of the learning set (Table 2), we selected the point on the ROC curve that was closest to the point (0,1) as the optimal threshold point. We also evaluated two other criteria: the minimum absolute difference between sensitivity and specificity, and the Youden index.[20] The cutoffs selected by these criteria were similar. Furthermore, the validation set provided sensitivity, specificity, and positive and negative predictive values similar to those of the learning set (Table 2). As a result, we combined the two sets and calculated sensitivity, specificity, and positive and negative predictive values by using standard methods. The results for the sensitivity, specificity, and positive and negative predictive values for the

Table 2. Sensitivity, Specificity, and Positive and Negative Predictive Value

Variable	Inhibin B ≤ 31 ng/L	FSH > 11.5 mIU/mL	Inhibin B:FSH ratio ≤ 2.52 pg/mIU
Learning data set (n = 140)			
Sensitivity	100.0	81.0	77.1
Specificity	44.3	73.2	72.9
Positive predictive value	55.2	68.1	66.1
Negative predictive value	100.0	84.5	82.3
Validation data set (n = 135)			
Sensitivity	100.0	74.5	73.2
Specificity	45.6	75.0	76.0
Positive predictive value	48.8	61.4	61.2
Negative predictive value	100.0	84.6	84.5
Combined learning and validation data sets			
Sensitivity	100.0	78.1	75.3
Specificity	45.0	74.1	74.5
Positive predictive value	52.1	65.1	63.8
Negative predictive value	100.0	84.6	83.5

Abbreviation: FSH, follicle-stimulating hormone.

learning data set, the validation data set, and the combined data set are provided in Table 2. An inhibin B value of ≤ 31 ng/L (Fig 2A), a FSH value of more than 11.5 mIU/mL (Fig 2B), and an inhibin B:FSH ratio of ≤ 2.52 pg/mIU (Fig 2B) were determined to be the optimum levels.

The scatterplot of the serum level of inhibin B and sperm concentration is shown in Figure 3, with Spearman's correlation coefficient $rs = 0.70$. Eighty-nine of 89 azoospermic individuals (100.0%)

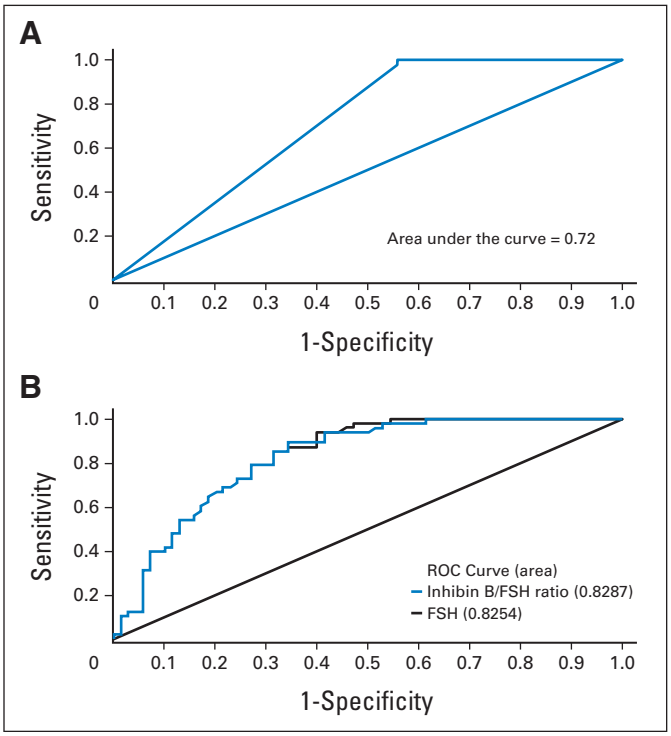

Fig 2. (A) Receiver operating characteristic (ROC) curve for inhibin B and (B) for follicle-stimulating hormone (FSH; solid line) and for inhibin B:FSH ratio (dashed line). AUC, area under the curve.

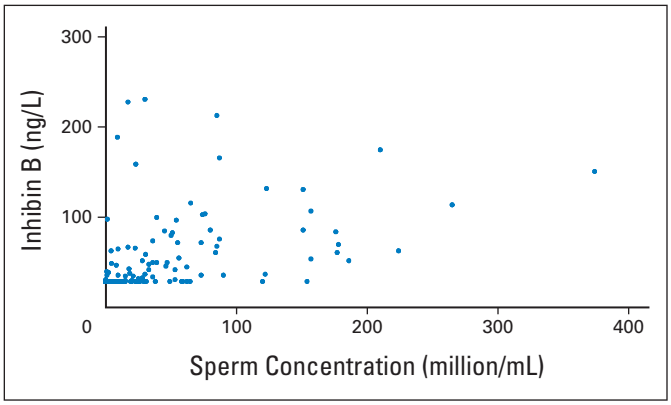

Fig 3. Scatterplot of inhibin B and sperm concentration values showing the positive relationship between these two values.

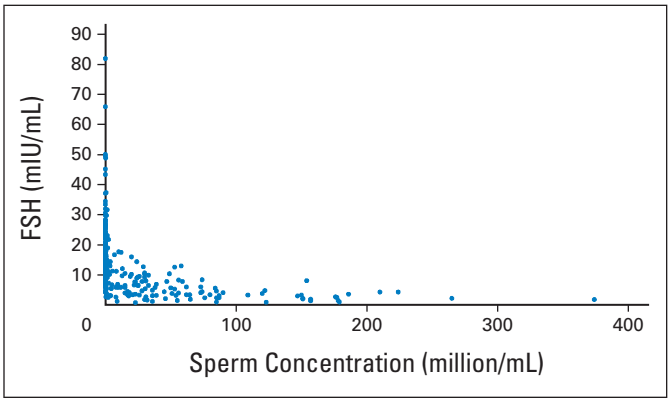

Fig 4. Scatterplot of follicle-stimulating hormone (FSH) and sperm concentration values showing the inverse relationship between these two values.

had a serum level of inhibin B ≤ 31 ng/L (Table 3). Of 149 individuals with oligospermia or a normal sperm count, 67 (45.0%) had a serum inhibin B level more than 31 ng/L. The specificity of serum inhibin B level ≤ 31 ng/L for identifying azoospermia was 45.0%, and the positive predictive value was 52.1% (Table 3).

A scatter plot of serum level of FSH and sperm concentration is shown in Figure 4 with Spearman's correlation coefficient $rs = -0.71$. Eighty-two (78.1%) of 105 azoospermic patients had a FSH level more than 11.5 mIU/mL (Table 3). One hundred twenty-six (74.1%) of those with oligospermia or a normal sperm count had a FSH level ≤ 11.5 mIU/mL. The specificity of a FSH level more than 11.5 mIU/mL for identifying azoospermia was 74.1%, and the positive predictive value was 65.1% (Table 2).

The optimal value for the inhibin B:FSH ratio for identifying participants with azoospermia was 2.52 pg/mIU. The ratio of inhibin B:FSH was not more useful for identifying participants with azoospermia than was FSH more than 11.5 mIU/mL. The specificity of the ratio ≤ 2.52 pg/mIU was 74.5%, and the positive predictive value was 63.8% (Table 2). The area under the ROC curves was 0.83 for FSH and 0.83 for the inhibin B:FSH ratio ($P = .31$; Fig 2B).

DISCUSSION

We have demonstrated that, although the serum level of inhibin B is directly and that of FSH is inversely correlated with sperm concentration, determination of the serum levels of neither inhibin B nor FSH, nor their ratio, is adequate for distinguishing between azoospermic and nonazoospermic long-term survivors of childhood cancer because of the lack of specificity and positive predictive value of both serum markers.

Table 3. Frequency of Decreased Inhibin B and Increased FSH Among Study Subjects With and Without Azoospermia

	Inhibin B (ng/L)		FSH (mIU/mL)		Inhibin B:FSH Ratio (pg/mIU)	
Characteristic	≤ 31	> 31	≤ 11.5	> 11.5	≤ 2.52	> 2.52
Azoospermia						
Yes	89	0	23	82	67	22
No	82	67	126	44	38	111

Abbreviation: FSH, follicle-stimulating hormone.

Previous investigations demonstrated a direct correlation between sperm concentration and inhibin B level in relatively small cohorts of long-term survivors of Hodgkin lymphoma treated before age 16 years (n = 21),[12] of Ewing or soft tissue sarcoma before age 22 years (n = 13),[21] and a variety of malignancies before age 16 years (n = 21 to 23).[13,22] Thomson et al[23] reported that basal inhibin B was significantly lower among azoospermic adult male survivors of childhood cancer (n = 7) than nonazoospermic adult male survivors of childhood cancer (n = 20).

Although the preceding publications demonstrated a significant correlation between the serum level of inhibin B and FSH and sperm concentration, only Romerius et al[14] previously examined the sensitivity, specificity, and positive and negative predictive values for these markers and azoospermia in survivors of childhood cancer. By using ROC analysis, they found that the optimal value for inhibin B was 50 ng/L, which was also the lower limit of the normal range for the inhibin B assay they used. When using this value, the sensitivity was 0.91 and the specificity was 0.90. The positive predictive value was 66% (95% CI, 47% to 81%), and the negative predictive value was 98% (95% CI, 93% to 100%).[14] In addition, they reported that the sensitivity of FSH for azoospermia was 0.96 and the specificity was 0.96 by using a FSH threshold of 10.9 IU/L. The positive predictive value was 50% (95% CI, 35% to 67%), and the negative predictive value was 99% (95% CI, 94% to 100%).[14]

The study by Romerius et al[14] included only 19 participants (14.7%) who had been treated with sterilizing doses and 40 (32.5%) who had been treated with nonsterilizing doses of cisplatin or alkylating agents with or without radiation therapy to cranial, supradiaphragmatic, and/or infradiaphragmatic treatment volumes and/or total-body irradiation. By contrast, all of the individuals evaluated in this study were exposed to potentially gonadotoxic treatment. In the study by Romerius et al,[14] only 23 (17.8%) of 129 participants were azoospermic compared with 105 (38.2%) of 275 in this study. The differences in the composition of the study populations, the larger number of events, and the larger size of the cohort may have contributed to the differences in specificity observed between the two studies.

We evaluated the inhibin B:FSH ratio based on the data of Andersson et al[24] who reported that an inhibin B:FSH ratio more than

23.5 ng/IU gave a sensitivity of 62% and specificity of 95% for identifying men of proven fertility in their study population. The ratio was more sensitive than either inhibin B or FSH for identifying men of proven fertility.[24] Our data suggest that the ratio is not more sensitive or specific for identification of azoospermic cancer survivors than either FSH or inhibin B. However, the limited recruitment of only 60.6% of the potentially eligible patients should be considered in the interpretation of our study findings. Notably, many of those who did not submit semen specimens had proven fertility (89 [49.7%] of 179).

Our results have important implications for counseling male long-term survivors of childhood cancer regarding their potential for fertility. Although the presence of a detectable level of inhibin B excluded azoospermia in the majority of men evaluated in this study, neither inhibin B nor FSH levels, individually or combined, should be used for advising men regarding the adequacy of spermatogenesis. All sexually active males should be instructed that, regardless of the levels of these biomarkers, adequate methods of contraception should be used if paternity is not a desired outcome of sexual activity. Moreover, because the return of spermatogenesis has been reported after prolonged periods of post-treatment azoospermia,[25-29] similar precautions should be exercised regardless of the results of a single semen analysis. Additional research is needed to identify a surrogate marker that has greater specificity and positive predictive value for sperm concentration than any of the currently available clinical biomarkers.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Daniel M. Green, William H. Kutteh, James L. Klosky, Monika L. Metzger, Fariba Navid, Melissa M. Hudson
Administrative support: Leslie L. Robison, Melissa M. Hudson
Collection and assembly of data: Raymond W. Ke, William H. Kutteh, Leslie L. Robison, Melissa M. Hudson
Data analysis and interpretation: Liang Zhu, Nan Zhang, Charles A. Sklar, Sheri L. Spunt, DeoKumar Srivastava, Leslie L. Robison, Melissa M. Hudson
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Howlader N, Noone AM, Krapcho M, et al: SEER Cancer Statistics Review, 1975-2008. 2011
2. Green DM, Kawashima T, Stovall M, et al: Fertility of male survivors of childhood cancer: A report from the Childhood Cancer Survivor Study. J Clin Oncol 28:332-339, 2010
3. Schover LR, Rybicki LA, Martin BA, et al: Having children after cancer. A pilot survey of survivors' attitudes and experiences. Cancer 86: 697-709, 1999
4. Oosterhuis BE, Goodwin T, Kiernan M, et al: Concerns about infertility risks among pediatric oncology patients and their parents. Pediatr Blood Cancer 50:85-89, 2008
5. Klosky JL, Randolph ME, Navid F, et al: Sperm cryopreservation practices among adolescent cancer patients at risk for infertility. Pediatr Hematol Oncol 26:252-260, 2009
6. Tsigkou A, Luisi S, Reis FM, et al: Inhibins as diagnostic markers in human reproduction. Adv Clin Chem 45:1-29, 2008
7. Anderson RA, Wallace EM, Groome NP, et al: Physiological relationships between inhibin B, follicle stimulating hormone secretion and spermatogenesis in normal men and response to gonadotrophin suppression by exogenous testosterone. Hum Reprod 12:746-751, 1997
8. Jensen TK, Andersson AM, Hjollund NH, et al: Inhibin B as a serum marker of spermatogenesis: Correlation to differences in sperm concentration and follicle-stimulating hormone levels—A study of 349 Danish men. J Clin Endocrinol Metab 82:4059-4063, 1997
9. Klingmüller D, Haidl G: Inhibin B in men with normal and disturbed spermatogenesis. Hum Reprod 12:2376-2378, 1997
10. Pierik FH, Vreeburg JT, Stijnen T, et al: Serum inhibin B as a marker of spermatogenesis. J Clin Endocrinol Metab 83:3110-3114, 1998
11. Kumanov P, Nandipati K, Tomova A, et al: Inhibin B is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility. Fertil Steril 86:332-338, 2006
12. van Beek RD, Smit M, van den Heuvel-Eibrink MM, et al: Inhibin B is superior to FSH as a serum marker for spermatogenesis in men treated for Hodgkin's lymphoma with chemotherapy during childhood. Hum Reprod 22:3215-3222, 2007
13. Lähteenmäki PM, Arola M, Suominen J, et al: Male reproductive health after childhood cancer. Acta Paediatr 97:935-942, 2008
14. Romerius P, Ståhl O, Moëll C, et al: High risk of azoospermia in men treated for childhood cancer. Int J Androl 34:69-76, 2011
15. Sklar CA, Constine LS: Chronic neuroendocrinological sequelae of radiation therapy. Int J Radiat Oncol Biol Phys 31:1113-1121, 1995
16. Hudson MM, Ness KK, Nolan VG, et al: Prospective medical assessment of adults surviving childhood cancer: Study design, cohort characteristics, and feasibility of the St. Jude Lifetime Cohort Study. Pediatr Blood Cancer 56:825-836, 2011
17. Robison LL, Mertens AC, Boice JD, et al: Study design and cohort characteristics of the Childhood Cancer Survivor Study: A multi-institutional collaborative project. Med Pediatr Oncol 38:229-239, 2002
18. Landier W, Bhatia S, Eshelman DA, et al: Development of risk-based guidelines for pediatric cancer survivors: The Children's Oncology Group Long-Term Follow-Up Guidelines from the Children's Oncology Group Late Effects Committee and Nursing Discipline. J Clin Oncol 22:4979-4990, 2004
19. World Health Organization (WHO): WHO Laboratory Manual for the Examination and Processing of Human Semen (ed 5). WHO, 2010
20. Pepe MS: The Statistical Evaluation of Medical Tests for Classification and Prediction. New York, NY, Oxford University Press, 2004
21. Williams D, Crofton PM, Levitt G: Does ifosfamide affect gonadal function? Pediatr Blood Cancer 50:347-351, 2008
22. van Casteren NJ, van der Linden GH, Hakvoort-Cammel FG, et al: Effect of childhood cancer treatment on fertility markers in adult male long-term survivors. Pediatr Blood Cancer 52:108-112, 2009
23. Thomson AB, Campbell AJ, Irvine DC, et al: Semen quality and spermatozoal DNA integrity in survivors of childhood cancer: A case-control study. Lancet 360:361-367, 2002
24. Andersson AM, Petersen JH, Jørgensen N, et al: Serum inhibin B and follicle-stimulating hormone levels as tools in the evaluation of infertile men: Significance of adequate reference values from proven fertile men. J Clin Endocrinol Metab 89: 2873-2879, 2004
25. Viviani S, Santoro A, Ragni G, et al: Gonadal toxicity after combination chemotherapy for Hodgkin's disease: Comparative results of MOPP vs ABVD. Eur J Cancer Clin Oncol 21:601-605, 1985
26. Meistrich ML, Chawla SP, Da Cunha MF, et al: Recovery of sperm production after chemotherapy for osteosarcoma. Cancer 63:2115-2123, 1999
27. Meistrich ML, Wilson G, Brown B, et al: Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing and soft tissue sarcomas. Cancer 70:2703-2712, 1992
28. Pryzant RM, Meistrich ML, Wilson G, et al: Long-term reduction in sperm count after chemotherapy with and without radiation therapy for non-Hodgkin's lymphomas. J Clin Oncol 11:239-247, 1993
29. Asbjornsen G, Molne K, Klepp O, et al: Testicular function after combination chemotherapy for Hodgkin's disease. Scand J Haematol 16:66-69, 1976

