

DNA damage in patients with untreated cancer as measured by the sperm chromatin structure assay

Hiroshi Kobayashi, M.D.,^a Kjersten Larson, M.S.,^c Rakesh K. Sharma, Ph.D.,^a David R. Nelson, M.S.,^b Donald P. Evenson, Ph.D.,^c Hiroshi Toma, M.D.,^d Anthony J. Thomas, Jr., M.D.,^a and Ashok Agarwal, Ph.D.^a

Center for Advanced Research in Human Reproduction and Infertility,
Urological Institute, Cleveland Clinic Foundation, Cleveland, Ohio

Objective: To examine the relationship between sperm chromatin defects, evaluated by sperm chromatin structure assay (SCSA) and semen characteristics in cryopreserved semen specimens from patients diagnosed with various types of cancer.

Design: Prospective study.

Setting: Andrology laboratory at a tertiary care hospital.

Patient(s): Cryopreserved semen samples from 12 healthy fertile men and 37 men diagnosed with cancer; testicular cancer (n = 20), Hodgkin's disease (n = 11), non-Hodgkin's disease (n = 4), and other neoplasm (n = 2).

Intervention(s): None.

Main Outcome Measure(s): The shift of green (native DNA) to red (denatured, single-stranded DNA) fluorescence in acridine orange-stained nuclei was measured and quantified using the expression ∞_t (red fluorescence / [red + green fluorescence] per cell). Sperm DNA damage was correlated with classical semen characteristics.

Result(s): Cancer patients as a group had significantly higher DNA damage when compared with controls. Specimens with high $\text{COMP}\infty_t$ values (percentage of sperm with denatured DNA) were present in all groups of cancer patients. No meaningful correlation was seen between the extent of DNA damage and classical semen measures.

Conclusion(s): DNA damage in spermatozoa is prevalent in the majority of cancer patients. SCSA provides important information about the biochemical integrity of sperm DNA in men with cancer before their treatment. (Fertil Steril® 2001;75:469-75. ©2001 by American Society for Reproductive Medicine.)

Key Words: DNA damage, testis cancer, Hodgkin's disease, non-Hodgkin's disease, SCSA

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Reprint requests: Ashok Agarwal, Ph.D., HCLD, Director, Andrology Research and Clinical Laboratories, Urological Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, A19.1, Cleveland, Ohio 44195 FAX: 216-445-6049; E-mail: agarwaa@ccf.org).

^a Center for Advanced Research in Human Reproduction and Infertility.

^b Department of Biostatistics and Epidemiology, Cleveland Clinic Foundation.

^c Olson Biochemistry Laboratories, South Dakota State University, Brookings, South Dakota.

^d Tokyo Women's Medical University, Department of Urology, Tokyo, Japan.

Testicular cancer, Hodgkin's disease, and leukemia are among the most common malignancies affecting men of reproductive age (1-5). In particular, the incidence of testicular cancer has increased during the last 4 to 5 years (6, 7). These cancers adversely affect sperm count and motility in prefreeze and postthaw specimens (8). As the treatment modalities for malignant diseases are improving, the effect of aggressive therapy on fertility is becoming more apparent (1).

Infertility is a major sequel of cancer treatment. Urologists and oncologists frequently provide referrals to a sperm bank for cancer patients before initiating chemotherapy, radiation therapy, or surgery. Infertility is associated with testicular cancer before any therapy is given (9,10). In about 52% of patients with testicular cancer and 40% of patients with other cancer, the total sperm count is significantly reduced at diagnosis and at treatment (11). Poor semen quality before freezing has been associated with poor postthaw outcome (2,12,13). In patients with testicular cancer, a germ cell defect is thought to be responsible for poor semen quality, although this defect has not been identified (14-16). Such a direct effect of the disease on semen quality in Hodgkin's disease has not been described. Although pregnancies and births have been reported using cryopreserved sperm from these patients (3,13,17,18), it is unclear whether spermatozoa from patients with Hodgkin's disease have decreased fertilization potential (2).

The structural organization of sperm DNA is vital for the proper functioning of the spermatozoa (19). It is not clear whether the cancer itself is capable of inducing changes in the genomic integrity of the spermatozoa. If the DNA is structurally intact, such patients can enroll in sperm banking and have increased success when using assisted reproductive procedures such as intracytoplasmic sperm injection (ICSI).

This study used the sperm chromatin assay (SCSA), used extensively to assess both animal and human fertility (20-22), as a descriptor of semen quality in cancer patients reflecting semen characteristics complementary to and beyond those of light microscopy assessment. First, we determined whether patients diagnosed with various types of cancer have higher incidence of DNA damage in their frozen ejaculates before treatment as determined by the SCSA. Second, we compared DNA damage data with conventional sperm characteristics in these patients.

MATERIALS AND METHODS

Selection of Subjects

The Institutional Review Board of the Cleveland Clinic Foundation approved this controlled prospective study measuring DNA damage in semen specimens from cancer patients that were banked before initiation of the treatment. Consent to use these specimens for research was obtained from patients who were no longer interested in continuing to bank their specimens, and notarized consent from family members was obtained for samples from the deceased patients. We studied samples from 37 cancer patients with the following diagnoses: testicular cancer (n = 20), Hodgkin's disease (n = 11), non-Hodgkin's disease (n = 4), and other neoplasms (n = 2). Patients were selected regardless of the stage of disease. Only those patients who had no history of chemotherapy or radiation therapy at the time of semen banking were included in the study. Twelve proven fertile donors who had fathered a child within the last 12 months were included as controls on the basis of a normal semen analysis, that is, volume \geq 2.0 mL; sperm count \geq 20 X 10⁶/mL; motility \geq 50%, and normal morphology \geq 30% according to the World Health Organization guidelines (23).

Assessment of Semen Characteristics

Semen specimens were collected by masturbation and into a plastic clinical specimen jar. Five μ L of liquefied semen was loaded on a 20- μ L MicroCell chamber (Conception Technologies, San Diego, CA). The samples were analyzed before and after cryopreservation with a Hamilton-Thorne Motion Analyzer (HTM version 10, IVOS. model, Hamilton-Thorne Research. Natick, MA) to assess various sperm motion characteristics.

Cryopreservation of Semen Specimens

TEST-yolk buffer with glycerol was used as a freezing medium for cryopreservation. A 5-mL vial of the freezing medium was thawed by incubating at 37°C for 30 minutes, and an aliquot equal to 25% of the original specimen volume was added to the specimen. This process were repeated four times to give a final 1:1 volume ratio of freezing medium to ejaculate. These samples were aliquoted into cryovials and held at -20°C for 8 minutes, placed in liquid nitrogen vapors (-79°C) for 2 hours, and transferred to liquid nitrogen for long-term storage.

Sperm Chromatin Structure Assay

Frozen semen samples were thawed in a 37°C water bath, and immediately diluted with TNE buffer (0.15 M NaCl, 0.01 M Tris, and 0.001 M EDTA; pH 7.4) to 1-2 x 10⁶ sperm cells per milliliter. Four hundred microliters of acid-detergent solution (0.08 M HCl, 0.15 M NaCl, 0.1% Triton X-100, pH 1.2) was added to 200 µL of the diluted sample. After 30 seconds, spermatozoa were stained by adding 1.2 mL of acridine orange (AO) staining solution containing 6 µg AO (chromatographically purified; Polysciences Inc., Warrington, PA) per milliliter of buffer (0.037 M citric acid, 0.126 M Na₂HPO₄, 0.0011 M EDTA [disodium], 0.15 M NaCl; pH 6.0; see References 24, 25). After 3 minutes of staining, fluorescence measurements were collected on 7,000 cells per sample using a Cytofluorograf flow cytometer (Ortho Diagnostic Inc, Westwood, MA) with a Lexel 100-mW argon ion laser, operated at 35 mW and interfaced to a Cicero data handling unit with PC-based Cyclops software (Cytomation, Fort Collins, CO).

The extent of DNA denaturation was quantified by the following calculated parameter: ∞_t ($\infty_t = \text{red}/[\text{red} + \text{green}]$ fluorescence; see Reference 26). Normal, native chromatin remains structurally intact and produces a narrow ∞_t distribution. DNA in spermatozoa with abnormal chromatin structure has increased red fluorescence (25, 27), with an ∞_t distribution that is broader, having a higher mean channel (X_{∞_t}) and a larger percentage of cells outside the main population of cells (COMP ∞_t). Standard deviation of ∞_t (SD ∞_t) describes the extent of chromatin structure abnormality within a population. The percentage of sperm with elevated green fluorescence (HGRN) reflects the degree of sperm chromatin condensation.

STATISTICAL ANALYSIS

Data were reported as the mean ± SE for a series of experiments using repeated-measures ANOVA. The pairwise comparisons from repeated-measures ANOVA were used to compare differences in all the variables for control and each cancer group and also between the testicular cancer and Hodgkin's disease groups. Spearman correlation coefficient was used to determine the correlation of semen parameters with the SCSA parameters (note: the SCSA is primarily a measure of DNA/chromatin integrity and secondarily provides information on the extent of chromatin condensation). A P value of <0.05 was considered significant using the SAS statistical software package (SAS Institute Inc., version 6.12, Cary, NC).

RESULTS

All 20 patients with testicular cancer had normal ejaculate volume. Of the patients in all cancer groups, 24.3% were oligospermic (<20 X 10⁶ sperm per milliliter), as defined by WHO criteria. Oligospermia occurred in 35% (n = 7) of the group of patients with testicular cancer and in 18.2% (n = 2) in the Hodgkin's disease group.

Prefreeze and Postthaw Semen Parameters in Different Cancer Groups

Table 1 summarizes the prefreeze and postthaw results of various conventional semen parameters measured in 12 proven fertile donors and 37 cancer patients. When prefreeze donor specimens were compared with those from all cancer patients, four characteristics differed significantly: sperm count, motility, straight-line velocity (VSL), and motile sperm count.

TABLE 1

Prefreeze and postthaw semen characteristics in healthy donors and cancer patients.

Group	Volume (mL)	Sperm concentration (x 10 ⁶ /mL)	Motility (%)	MSC (x 10 ⁶ /mL)	VSL (µm/s)	LIN (%)	ALH (µm)
Control (n = 12)							
Prefreeze	2.8 ± 0.4	118.0 ± 14.4	71.9 ± 3.0	58.4 ± 3.6	55.0 ± 3.4	23.1 ± 6.8	3.1 ± 0.2
Postthaw	-	--	46.2 ± 3.1	36.2 ± 2.3	29.6 ± 2.6	12.9 ± 4.9	2.2 ± 0.2
All cancer (n = 37)							
Prefreeze	3.4 ± 0.2 (P = 0.08)	83.3 ± 23.1 (P = 0.001)	47.2 ± 3.6 (P = 0.0007)	43.1 ± 16.1 (P = 0.0004)	37.8 ± 2.7 (P = 0.001)	16.5 ± 3.8 (P = 0.07)	2.8 ± 0.3 (P = 0.09)
Postthaw	-	--	24.1 ± 2.0 (P < 0.0001)	12.1 ± 3.5 (P < 0.0001)	29.2 ± 2.0 (P = 0.003)	14.8 ± 3.7 (P = 0.04)	2.5 ± 0.3 (P = 0.81)
Testicular cancer (n = 20)							
Prefreeze	3.5 ± 0.2 (P = 0.10)	29.5 ± 5.1 (P < 0.0001)	47.4 ± 4.8 (P = 0.002)	14.0 ± 3.1 (P < 0.0001)	42.4 ± 3.8 (P = 0.003)	15.6 ± 5.2 (P = 0.15)	2.6 ± 0.4 (P = 0.10)
	--	--	24.4 ± 2.3 (P < 0.0001)	3.7 ± 0.8 (P < 0.0001)	36.6 ± 3.2 (P = 0.02)	12.6 ± 5.2 (P = 0.13)	2.8 ± 0.4 (P = 0.97)
Hodgkin's (n = 11)							
Prefreeze	3.2 ± 0.5 (P = 0.32)	109.9 ± 66.0 (P = 0.32)	49.5 ± 7.8 (P = 0.02)	70.2 ± 57.5 (P = 0.32)	39.5 ± 5.3 (P = 0.03)	5.3 ± 0.6 (P = 0.01)	3.2 ± 0.7 (P = 0.05)
Postthaw	-	-	23.7 ± 4.3 (P = 0.06)	20.4 ± 1.9 (P = 0.06)	29.5 ± 3.6 (P = 0.003)	4.9 ± 0.3 (P = 0.001)	2.3 ± 0.2 (P = .38)
Non-Hodgkin's (n = 4)							
Prefreeze	2.9 ± 0.7 (P = 0.23)	117.3 ± 108.2 (P = 0.79)	46.5 ± 1.6 (P = 0.004)	42.1 ± 4.9 (P = 0.02)	43.1 ± 6.1 (P = 0.05)	13.2 ± 10.4 (P = 0.13)	2.8 ± 0.8 (P = 0.25)
Postthaw	-	-	29.1 ± 4.3 (P = 0.01)	14.7 ± 7.7 (P = 0.07)	37.3 ± 3.5 (P = 0.06)	13.7 ± 9.1 (P = 0.19)	3.1 ± 1.0 (P = .09)
Other neoplasm (n = 2)							
Prefreeze	3.1 ± 0.6	61.9 ± 31.1	66.4 ± 11.2	43.0 ± 25.2	36.0 ± 13.1	52.2 ± 1.9	3.8 ± 0.3
Postthaw	-	-	39.2 ± 9.7	11.9 ± 7.2	34.1 ± 9.2	52.2 ± 8.0	3.5 ± 0.2
Testicular vs. Hodgkin's							
Prefreeze	(P = 0.98)	(P = 0.003)	(P = 0.72)	(P = 0.13)	(P = 0.77)	(P = 0.25)	(P = 0.16)
Postthaw	-	-	(P = .83)	(P = 0.07)	(P = 0.25)	(P = 0.20)	(P = 0.79)

Note: Values are mean ± SE. MSC = motile sperm count; LIN = linearity; ALH = amplitude of lateral sperm head displacement. Kobayashi. DNA damage in untreated cancer patients. Fertil Steril 2001.

The testicular cancer group differed significantly from controls in prefreeze sperm count, motility, VSL, and motile sperm count and in postthaw motility, VSL, and motile sperm count. The Hodgkin's group differed significantly in prefreeze motility, VSL, and linearity and in postthaw motility, VSL, and linearity. The non-Hodgkin's group differed from controls in prefreeze motility and motile sperm count and in postthaw motility (Table 1). Within the cancer patients, the testicular cancer group had significantly lower sperm counts than those with Hodgkin's disease.

SCSA Variables in Fertile Men and Cancer Patients

The mean value of %COMP α_t in the control group was 9.4%, with an upper limit of 17.5. The COMP α_t values in the control group ranged from a low of 6.4 to a high of 17.5%. Similarly, the HGRN ranged from a low of 3.9% to a high of 24.4% (Table 2). These values were consistent with high fertility potential (21). Table 3 shows that in the cancer group, chromatin integrity was highly variable. Cancer patients as a group were significantly different from controls in COMP α_t , X α_t , and SD α_t , (Figure 1). The table also shows that testicular cancer patients had significantly more spermatozoa DNA damage than, did controls. However, HGRN in the testicular cancer group was higher but not significantly different from the control value. A wide spread was seen in the distribution of COMP α_t , values greater than 25%. In 10 patients, the COMP α_t , value was less than 16%. Increased DNA stainability (HGRN \geq 15%) was seen in eight (40%) of the patients (Table 2).

The non-Hodgkin's disease group also differed significantly from controls in COMP α_t , X α_t , and SD α_t (Table 3). In one patient, the COMP α_t value was as high as 60%. Increased DNA stainability (HGRN > 15%) was seen in only one patient (25%; Table 2).

TABLE 2

Pretreatment SCSA results (COMP α_t and HGRN) in proven fertile donors and in patients with different types of cancer.

Donor No:	(%) COMP α_t	(%) HGRN
Proven fertile donors		
1	8.8	3.9
2	6.4	4.2
3	8.2	6.6
4	16.4	11.1
5	7.2	13.4
6	7.2	10
7	11.2	5
8	9.8	7.5
9	12.5	12
10	13	6.4
11	10.5	24.4
12	17.5	5.8
Testicular cancer		
1	12.2	7.1
2	23.2	16.7
3	15.6	33.4
4	15.6	11.3
5	55.9	8.2
6	46.8	11.6
7	21	7.2
8	21.8	7.1
9	13.9	17.8
10	26.2	13.6
11	14.9	21.5
12	25.9	15.2
13	13	6.2
14	28.5	24.1
15	12.2	10.9
16	4.3	12.6
17	9.4	17.3
18	15.1	9.5
19	51.2	2.9
20	37.3	41.1
Hodgkin's disease		
1	6.7	10.6
2	22.8	7.8
3	11.9	23.5
4	21.1	8.7
5	34.6	9.7
6	8.9	9.1
7	20.1	5.2
8	12.5	12.1
9	43	10.9
10	18.4	7.2
11	8	6
Non-Hodgkin's disease		
1	13.7	10.6
2	13.1	11.8
3	60.4	4.1
4	13.6	17.2
Other neoplasm		
1	10.6	15.9
2	19.2	21.7

The Hodgkin's disease group differed significantly from controls in X α_t and SD α_t . COMP α_t values greater than 20% were seen in five patients, the greatest being 43% (Table 2). Increased DNA stainability (HGRN > 15%) was seen in one (9%) of the patients (Table 2). Hodgkin's disease patients did not differ significantly from testicular cancer patients.

When values for the Hodgkin's and non-Hodgkin's disease groups were combined, they differed significantly from controls in X α_t , COMP α_t , and SD α_t (Table 3). COMP α_t values greater than 20% were seen in six patients (Table 2).

The group with other neoplasms did not differ significantly from controls in any SCSA parameters.

Relationships Between SCSA and Conventional Sperm Parameters

In prefreeze and postthaw specimens, the SCSA findings and the conventional semen quality measures showed no clinically significant correlations.

DISCUSSION

The purpose of this study was to determine the variability of SCSA data across a normal healthy population and compare it with that of cancer patients. In our study, cancer patients had a wide range of chromatin integrity, from excellent (COMP α_t = 4%) to very poor (COMP α_t = 62%; Table 3). Yet overall, COMP α_t values in cancer patients were significantly higher than those in controls. Samples with normal and abnormal COMP α_t values were seen in all groups. Earlier reports have also verified that SCSA values rise in spermatozoa from cancer patients (28, 29).

Previous studies (21) demonstrated that COMP α_t was the best SCSA parameter to predict whether a couple would not become pregnant based on logistic regression results. The threshold currently set for considering a semen sample compatible with fertility is 30% of the cells in the population showing denaturation (COMP α_t). Fossa et al. (28) reported 16% COMP α_t value as the upper threshold for the control samples. However, this limit was not verified from in vivo results and cannot be compared with the 30% COMP α_t , which was proven to predict in vivo infertility where no pregnancies were produced in IVF cycles that used sperm with \geq 30% COMP α_t values in the semen sample (21). Therefore,

TABLE 3

Sperm chromatin structure assay results in cancer patients and healthy donors.

Group	$X\alpha_\tau$	$SD\alpha_\tau$	$COMP\alpha_\tau$	HGRN
Control (n = 12)	226.2 ± 17.7	136.7 ± 13.3	10.7 ± 3.5	9.2 ± 1.8
Testicular cancer (n = 20).	270.6 ± 14.5 (<i>P</i> = 0.04)	187.6 ± 10.5 (<i>P</i> = 0.005)	21.9 ± 10.7 (<i>P</i> = 0.02)	13.5 ± 1.4 (<i>P</i> = 0.06)
Non-Hodgkin's (n = 4)	307.0 ± 30.7 (<i>P</i> = 0.02)	203.3 ± 23.1 (<i>P</i> = 0.02)	27.8 ± 6.0 (<i>P</i> = 0.02)	11.0 ± 3.1 (<i>P</i> = 0.62)
Hodgkin's (n = 11)	274.1 ± 18.8 (<i>P</i> = 0.05)	187.9 ± 14.0 (<i>P</i> = 0.01)	20.8 ± 3.7 (<i>P</i> = 0.05)	9.8 ± 1.9 (<i>P</i> = 0.81)
Non-Hodgkin's + Hodgkin's (n = 15)	283.1 ± 160.0 (<i>P</i> = 0.01)	192.0 ± 11.9 (<i>P</i> = 0.03)	22.7 ± 3.1 (<i>P</i> = 0.01)	10.1 ± 1.6 (<i>P</i> = 0.70)
Other neoplasm (n = 2)	259.3 ± 43.4	185.0 ± 32.7	14.9 ± 8.5	18.8 ± 4.4
All cancer patients (n = 37)	275.2 ± 10.3 (<i>P</i> = 0.01)	189.3 ± 7.6 (<i>P</i> = 0.001)	21.9 ± 2.0 (<i>P</i> = 0.007)	12.4 ± 1.1 (<i>P</i> = 0.13)
Testicular vs Hodgkin's	(<i>P</i> = 0.90)	(<i>P</i> = 0.98)	(<i>P</i> = 0.84)	(<i>P</i> = 0.14)

Note: Values are mean ± SE. All *P* values indicate a statistically significant difference when compared with the control value (*P* < 0.05 was considered statistically significant). $X\alpha_\tau$ = mean of α_τ population; $SD\alpha_\tau$ = standard deviation of α_τ population.

Kobayashi. DNA damage in untreated cancer patients. Fertil Steril 2001.

$COMP\alpha_\tau$ values in the semen sample might be indicative of more extensive DNA damage within the sperm population, described as the "tip of the iceberg" effect (21). That is, although only a fraction of the sperm had strand breaks that resulted in single-stranded DNA after exposure to the SCSA acid treatment, the remaining sperm may still have some uncompensated DNA damage, despite the lack of expression of single-stranded DNA under these assay conditions. The $SD\alpha_\tau$ adds important information by describing the variation from the mean. In our study, $SD\alpha_\tau$ was significantly higher in all cancer groups except the "other neoplasm" group. A high $SD\alpha_\tau$ suggests that a large quantity of chromatin in the sample is damaged, even though no single cell may have crossed the threshold to be counted as a DNA-denatured cell. This damage may be sufficient to impair fertility or negatively affect pregnancy outcome (21). The α_τ values probably reflect a variety of anomalies during the complex spermiogenic pathway, eventually leading to incorrect chromatin packaging. Fertile patients have a much more homogenous SCSA pattern than do subfertile patients (21).

Chromatin alterations measured by SCSA have been found to parallel the pattern of dominant lethal mutations, which are most likely a consequence of chromosomal breaks (30). Thus, the SCSA appears to detect early stages of chromatin alterations that most likely lead to chromosome breaks. High SCSA values are not sufficient for fertility, which also requires good sperm motility, morphology, acrosome integrity, and so on. However, poor SCSA values predict subfertility and infertility.

The SCSA appears to be an independent assay capable of diagnosing a pathological problem in sperm that is not detectable by any other method (31-35). Our results support this, showing that SCSA data have low correlation with conventional semen characteristics. Defective spermiogenesis, manifested by morphologically abnormal sperm cells and infertility, is characterized by increased sensitivity of DNA in situ to denaturation (27, 36). Sperm cells with DNA highly susceptible to low-pH denaturation are considered dead in terms of their reproductive capacity, despite having intact cell membranes and mitochondria and even good motility. These spermatozoa therefore resemble somatic cells programmed for apoptosis, which have DNA that is highly susceptible to denaturation but also have intact cell membranes, mitochondria, and lysosomes (37-41). Eliminating the reproductive capacity of sperm cells with one or a few potentially mutagenic DNA lesions is clearly evolutionarily advantageous because it would prevent congenital malformations.

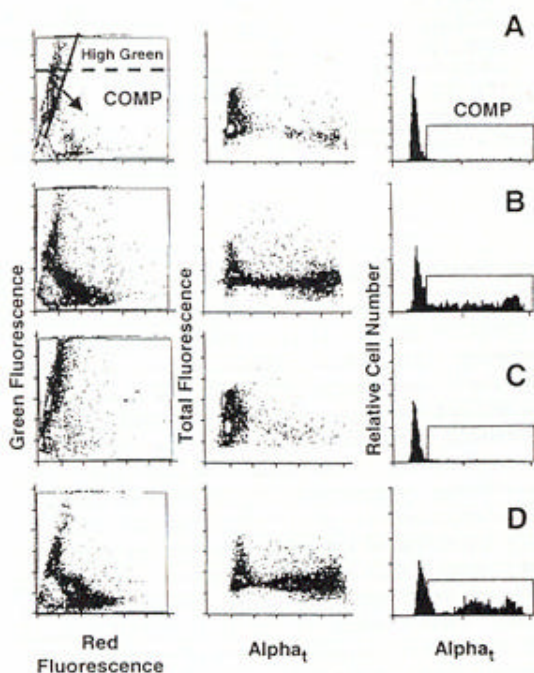
We found negative correlations or lack of significant clinical correlations between various SCSA measures and conventional semen characteristics, measured by computer-assisted semen analyzer. The main correlate of $COMP\alpha_\tau$ was motility in the overall cancer group. A marginal relationship between DNA damage and semen quality, such as we found, has been reported by other laboratories (42-44). A possible explanation for this could

be because of differences in the composition of the study groups, use of fresh versus frozen semen samples, and whether any sperm preparation protocols were employed.

The study also provides a strong rationale for andrology clinics to measure SCSA in all or selected semen samples before counseling patients in fertility issues, especially expensive assisted-reproductive procedures. SCSA is a rapid and statistically robust technique, allowing unbiased assessment of fluorescence colors. It avoids heterogenous staining conditions associated with cell crowding and uneven slide surfaces. SCSA test results are related to both in vivo and in vitro human fertility potential (45). Of importance to the andrology clinic are those cases where the classical semen criteria are within normal ranges but the SCSA values are poor. An ejaculate with little or no motility but with good SCSA results might be appropriate for ICSI. On the other hand, a sample with good motility and morphology but poor SCSA may not be useful; obtaining a testicular sample from this patient might be the best option.

FIGURE 1

Representative sperm chromatin structure assay data from (A) fertile donor, (B and C) testicular cancer, (D) non-Hodgkin's lymphoma patients. Cytograms in the left column show green versus red fluorescence of 7,000 spermatozoa. Cytograms of total fluorescence versus alphas are in the middle column. Alpha_t histograms with cells outside main population (COMP α_t regions) are shown in the right column. Note the increased DNA denaturation represented by the increased red fluorescence and COMP α_t in one of the two testicular patients (B) and in the non-Hodgkin's lymphoma patient (D). The region for high green fluorescence is indicated in the upper left-hand cytogram.



The integrity of sperm nuclear DNA is of paramount concern for the successful transmission of a competent paternal genome to the oocyte (46). High levels of sperm chromatin defects may be associated with decreased fertilization rates in assisted fertilization techniques (47-49). The miscarriage rate may also be higher with assisted fertilization (50), which could be consistent with the use of genomically compromised spermatozoa that conferred irreparable DNA damage to the embryo.

One of the main limitations of our study was we did not collect data on pregnancy outcome and that thus, we cannot contribute to this debate. In addition, we cannot say whether ICSI passed along genetic damage predisposing offspring to disease that does not manifest itself until later life, such as childhood cancer (51-53).

In men with cancer, the extent of DNA damage may help determine how semen should be cryopreserved before therapy begins. Specimens with high sperm concentration and motility and low levels of DNA damage could be preserved in relatively large aliquots suitable for intrauterine insemination (IUI). If a single specimen of good quality is available, then it should be preserved in multiple small aliquots suitable for IVF or ICSI.

For ICSI, deficiencies in sperm motility, zona penetration, and oolemma binding become irrelevant. Chromatin packaging assessments may be a more accurate method of predicting the fertilization potential of sperm used for ICSI. Thus, SCSA could be added to the sequential diagnostic steps used in assessing patients with malefactor infertility.

Of interest, in our study, the median sperm concentration was only 10 million per milliliter,

whereas the median in the general population is 50 million per milliliter or higher (54). Potential causes for oligospermia include preexisting defects in spermatogenesis, a possible history of cryptorchidism, hormone production by the tumor, antisperm antibodies, possible contralateral or intraepithelial germ cell neoplasia, and general stress associated with illness (4, 9, 55-56).

In conclusion, the result of SCSA provides important information about the biochemical integrity of sperm DNA in men with cancer before they receive treatment. Optimal sperm chromatin packaging seems necessary for full expression of the male fertility potential. Good or poor semen quality as defined by classical semen measures may not always reflect the DNA integrity in any given specimen. It seems prudent to evaluate the semen of interested patients before deciding for or against cryopreservation or ICSI. Our results may shed some light on the reasons for the low success rate of ICSI in cancer patients with normal semen characteristics.

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