NOWOTWORY Journal of Oncology • 2004 • volume 54
Number 2 • 100–107

Original papers

The effect of chemotherapy on numerical aberrations of spermatozoal chromosomes in testicular cancer patients

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Introduction. The incidence of germinal cell tumours of the testes reaches its peak in men between 20 and 40 years of age, constituting some 95% of all testicular malignancies. During the last 20 years the results of treatment of testicular malignancies have improved dramatically, the cure rate reaching 95% in patients staged as grades I and II and approximately 80% in patients staged as grade III.

The aim of the study was to evaluate the frequency of aneuploidy of chromosomes 13, 18, 21, X and Y in the seminal smears of patients with testicular cancer and in healthy controls, and then to analyze chromosomal numerical aberrations of chromosomes 13, 18, 21, X and Y after chemotherapy.

Material. The material constituted of 20 men with testicular cancer, who underwent evaluation of seminal smears for the frequency of chromosomal aberrations (13, 18, 21, X and Y) prior to and 30 months after the completion of chemotherapy (chth). During chemotherapy the patients received two courses of carboplatin or two to six courses of BEP (cis-platin, etoposide and bleomycin).

Results. We found no differences in the frequency of chromosomal numerical aberrations among the healthy men and the testicular cancer patients both prior to, and after the completion of chemotherapy; the cells with chromosomal numerical aberrations accounting for 1.76%, 2% and 2.09%, respectively.

Among the aneuploid spermatozoa we found hypohaploid, hyperhaploid and diploid cells. The ratio of each of the cell types was, respectively, 0.40%; 0.40% and 0.45% in the control group; 0.73%, 0.91% and 0.97% in the "prior to chth" group and 0.63%; 0.69% and 0.67% in the "post chth" group.

Autosomal (13, 18 and 21) disomy was observed in a total of 0.64% of spermatozoa of the control group subjects and in 0.74% and 0.83% of the study group prior to and after chth, respectively.

The frequency of nullsomy of chromosomes 13, 18 and 21 was 0.29% in the control group, 0.24% in the "prior to chth" group and 0.17% in the "post chth" group.

The X/Y ratio was found to be 50%/50% in all the three groups. The frequency of X and Y nullsomy was 0.14% in the control group, 0.19% in the "prior to chth" group and 0.13% in the "post chth" group. In the control group the diploid spermatozoa were discerned as XX in 0.05%; as XY in 0.49% and as YY in 0.09%. In the studied group "prior to chth" the same values reached 0.09%; 0.5% and 0.07% respectively, while in the "post chth" group they reached 0.09%; 0.49% and 0.07%, respectively.

In germinal cell tumour patients 30 months after platinum-based chemotherapy the risk of producing a child with 13, 18, 21, X or Y chromosome aneuploidy of the father's origin is low.

Conclusions. 1. Aneuploidy of chromosomes 13, 18, 21, X and Y is equally frequent in healthy males and in germinal cell tumour patients. 2. Platinum-based chemotherapy does not influence the frequency of aneuploidy of chromosomes 13, 18, 21, X and Y in the spermatozoa of germ cell tumour patients 30 months after treatment completion.

Wpływ chemioterapii na aberracje liczbowe chromosomów plemnikowych u chorych na nowotwory jądra

Największy odsetek zachorowań na nowotwory zarodkowe jądra występuje u mężczyzn w wieku 20-40 lat. Nowotwory te stanowią 95% wszystkich nowotworów jądra.

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Study financed by a grant from the State Committee for Scientific
Research No. 4PO5CO5112

W ostatnich dwudziestu latach radykalnie poprawiły się wyniki leczenia chorych na nowotwory złośliwe jądra. Obecnie odsetek wyleczeń sięga 90% w pierwszym i drugim stopniu zaawansowania oraz około 80% w trzecim stopniu zaawansowania.
Cel. Celem pracy była ocena częstości występowania aneuploidii chromosomów 13, 18, 21, X, Y w rozmazach plemnikowych grupy kontrolnej i grupy chorych na nowotwór jądra przed leczeniem. W pracy podano również analizę wpływu chemioterapii na występowanie aberracji liczbowych chromosomów 13, 18, 21, X, Y.
Materiał. Materiał stanowiło 20 mężczyzn, którym zbadano częstość aberracji chromosomalnych (13, 18, 21, X, Y) w nasieniu pobranym przed i 30 miesięcy po zakończeniu chemioterapii. Chorzy leczeni byli dwoma kursami karboplatyny lub cisplatyną, etopozydem i bleomycyną (BEP) w schemacie dwa, trzy, cztery lub sześć kursów BEP.
Wyniki. Nie stwierdzono różnic w częstości występowania zaburzeń liczby chromosomów plemnikowych w grupie zdrowych mężczyzn i w grupie chorych przed i po leczeniu. Komórki wykazujące zaburzenia liczby chromosomów stanowiły 1,76% w grupie kontrolnej, 2% w grupie przed chemioterapią i 2,09% po chemioterapii.
Wśród plemników aneuploidalnych wyróżniono komórki hipohaploidalne (k.hipo), hiperhaploidalne (k.hiper), diploidalne (dip). Odsetek poszczególnych rodzajów komórek w grupie kontrolnej oraz w grupie chorych przed i po chemioterapii wynosił odpowiednio: k.hipo – 0,40%, k.hiper – 0,40%, dip – 0,45%, k.hipo – 0,73%, k.hiper – 0,91%, dip – 0,97%, i k.hipo – 0,63%, k.hiper – 0,69%, dip – 0,67%.
Odsetek disomii autosomów (13, 18, 21) obserwowano ogółem w 0,64% plemników w grupie kontrolnej, oraz 0,74% i 0,83% plemników w grupie badanej przed i po chemioterapii.
Odsetek nullosomii chromosomów 13,18,21 w grupie kontrolnej oraz przed i po chemioterapii wynosił 0,29%, 0,24%, 0,17%.
W grupie kontrolnej oraz w grupie chorych przed i po chemioterapii stosunek X/Y wynosił 50% / 50%. Odsetek nullosomii chromosomów X lub Y wynosił odpowiednio 0,14%, 0,19%, 0,13%. W grupie kontrolnej plemniki z diploidalnym kariotypem, zawierającym chromosomy XX, występowały w 0,05%, chromosomy XY w 0,49% i chromosomy YY w 0,09%. W grupie chorych przed i po chemioterapii wartości te wynosiły odpowiednio 0,09%, 0,5%, 0,07% i 0,09%, 0,49%, 0,07%.
W grupie chorych na nowotwór jądra po upływie 30 miesięcy od zastosowanego leczenia preparatami platyny ryzyko urodzenia dziecka z aneuploidią chromosomów 13, 18, 21, X, Y pochodzenia ojcowskiego jest niskie.
Wnioski. 1. Aneuploidia chromosomów 13, 18, 21, X, Y występuje równie często w grupie zdrowych mężczyzn, jak i chorych na nowotwór jądra. 2. Chemioterapia preparatami platyny nie wpływa na częstość aneuploidii chromosomów 13, 18, 21, X, Y w plemnikach chorych na nowotwór jądra w 30 miesięcy po zakończeniu leczenia.

Key words: testicular cancer, chemotherapy, chromosomal aberrations
Słowa kluczowe: nowotwór jądra, chemioterapia, aberracje chromosomów

Introduction

In 1996 564 new cases of germinal cell tumours were diagnosed in Poland [1], their incidence listing them as the eighteenth most common malignancy and recognized as the 29th most common cause of death [1]. The peak of incidence is noted in subjects between 20 and 40 years of age and in those over the age of 60 [2]. Germinal cell tumours account for 95% of all testicular malignancies [2].

During the last 20 years the results of treatment of testicular malignancies have improved dramatically. In the beginning of the 1970's the cure rate hovered around 10%, while at present it reaches 95% in patients in the first and second stage of the disease and approximately 80% in patients in the third stage [3-5].

Young germinal cell tumour survivors (aged between 18 and 35 years) more and more often state their will to father children, and therefore both they, and their partners, seek to know their ability to produce healthy offsprings. Oncolytic drugs are rightly perceived as serious mutagens, while cells undergoing rapid division, such as those of the spermatogenetic epithelium, are at a greater risk of damage of their genetic apparatus. This is of extreme importance, because the damaged genetic material may find its way into reproductive cells, thus causing reproductive disturbances (embryonic death or congenital defects in the offspring). Because the spermatogenic epithelium originates from stem cell division, any genetic defect at this latter level may continue to be reproduced, thus causing repeated reproduction failures [6-8].

Among all the cells taking part in spermatogenesis the spermatogoniums and the primary spermatocytes in the preleptoten period are most likely to be affected by cytostatic drugs, as they undergo rapid divisions [10-12]. On the other hand, DNA and the chromosomes of secondary spermatocytes and spermatids are less prone to damage, as they do not divide so fast.

The DNA of spermatogoniums undergoes damages throughout the entire period of chemotherapy. This leads to azoospermia. Once chemotherapy is stopped the entire population of the reproductive cells should be restored over a period of 12 weeks. Unfortunately, in those patients in whom platinum derivatives were applied for testicular cancer the semen returns to its normal parameters only after some 2-3 years. We are not yet aware of the reasons for this delay. It is necessary to stress that permanent or transient oligo- or azoospermia depends on the type of drug, its dosage and the duration of treatment. Abnormalities of the spermatozoal DNA may affect fertilization and embryonic vitality and development; they may also influence the probability of

genetic disorders in the offspring. Spermatozoal DNA may now be evaluated owing to the development of cytometric methods (flow cytometry or imaging cytometry). These methods have revealed that abnormal DNA content may cause disorders in the structure and mobility of spermatozoa.

Studies aimed at discerning chromosomal aberrations within the spermatozoa have been conducted for a number of years. First reports of chromosomal aneuploidy in human gametes were published in 1980 [13].

Classical cytogenetic studies are conducted on chromosomes in the metaphase, and their analysis demands differential staining. At present we use either striatic analysis or the fluorescent *in situ* hybrydization (FISH) techniques, which allow to analyze chromosomal aberrations in the metaphase and also in interphase nuclei.

Chromosomal aberrations may be evaluated in human spermatozoa in the course of analysis of hamster oocyte penetration (Yanagimachi) or with the use of the FISH technique. It must be stressed that all these studies were carried out on very small groups of subjects (4-5 persons) and cannot form the base for definite conclusions.

Aims of the study

1. To assess the frequency of aneuploidy of chromosomes in 13, 18, 21, X and Y in seminal smears of testicular cancer patients as compared to healthy controls.
2. To evaluate the impact of systemic platin-based chemotherapy on the frequency of numerical aberrations of chromosomes 13, 18, 21, X and Y.

Material

The material included twenty patients with testicular cancer in whom we analyzed the frequency of chromosome 13, 18, 21, X and Y aberrations in spermatozoa from seminal samples obtained before, and 30 months after the completion of chemotherapy. The results were compared with results observed in the control group, which consisted of five healthy, fertile men between the ages of 20 and 30. Table I presents patient data – the number of spermatozoa present in the samples taken before and after chemotherapy, the type of applied chemotherapy, the histopathological types of the tumours and the stage of advancement of the tumours.

Method

Every patient underwent routine analysis of the ejaculate and ultrasonographic examination of the healthy testis both before chemotherapy and 30 months after its completion. After analysis the seminal sample was preserved in liquid nitrogen until the time of cytogenetic examination using the FISH technique. The ejaculates were obtained in the course of masturbation (at least 3 days after previous ejaculation), and routine examination was performed acc. to WHO standards after complete liquefaction [14]. Morphology of the spermatozoa was assessed after Pappenheim staining. The minimal number of examined spermatozoa per sample was 200. We applied the University of Minnesota modified system of clinical staging for germinal tumours of the testes. The results of the general examination of seminal samples of testicular cancer patients (obtained both prior to and after orchidectomy) were compared with analogous results from samples obtained from five healthy individuals.

Table I. Data of 20 testicular cancer patients in whom the frequency of chromosomal aberrations was analyzed (incl: number of spermatozoa, tumour pathology, grade of advance, type of chemotherapy)

Pt. number	Age (yrs.)	A	B	Pathology	Grade	Type of chth.
1	31	57	70	Non-S	IIC	3xBEP
2	22	56	28	Non-S	1H	2xBEP
3	45	62	140	S	1H	2xkarbo
4	32	87	19	Non-S	IIIA	4xBEP
5	20	19.5	4	Non-S	IIIB	4xBEP
6	30	84	50	Non-S	IIB	3xBEP
7	18	225	85	Non-S	1H	2xBEP
8	22	4.7	15	Non-S	IIIA	4xBEP
9	34	37.5	33	Non-S	IIIA	4xBEP
10	22	3.7	20	Non-S	IIA	3xBEP
11	39	34	10	S	IIIA	4xBEP
12	28	12	18	S	1H	2xkarbo
13	22	49	15	S	IIB	3xBEP
14	23	7	10	Non-S	IIIB	4xBEP
15	26	20	12	Non-S	1H	2xkarbo
16	30	5.6	39	Non-S	1H	2xBEP
17	30	41	18	S	1H	2xkarbo
18	20	30	70	Non-S	1H	2xkarbo
19	28	6.7	14	Non-S	1H	2xkarbo
20	22	21	18	S	IIA	3xBEP

A – number of spermatozoa in 1 ml of semen before chemotherapy x 10^6
B – number of spermatozoa in 1 ml of semen after chemotherapy x 10^6
Grade – grade of clinical advancement
S – seminoma
Non-S – non-seminoma
BEP – bleomycin, cisplatin, etopozyd
Karbo – carboplatin

The following chemotherapy regimes were applied:
1. Two courses of carboplatin – dose: 400 mg/m^2
2. BEP – cis-platin: 100 mg/m^2; fractionated doses, day 1-3 or 1-5
   etoposide: 500 mg/m^2; fractionated doses, day 1-3 or 1-5
   bleomycin: 30 u.; days 2, 8, 15 (21-day cycle)

In a 2-3-4 and 6 BEP cycles regime.

The MultiVision PGT test

In the course of the cytogenetic studies we used the MultiVysion PGT test (VYSIS) for the simultaneous detection of aneuploidy of chromosomes 13, 18, 21, X and Y within decondensed spermatozoal heads.

The seminal samples were centrifuged (1700 revolutions/5 minutes). The sediment was then suspended in PBS (buffered solution of saline with calcium chloride and magnesium chloride; pH 7) in a temperature of 37°C. After supernatant decantation cytogenetic specimens were prepared. They were dried on plates heated to 60°C and then underwent 20 minute fixation in cooled Carnoy solution (3:1 methyl alcohol and glacial acetic acid) in vessels used for histological staining. The spermatozoal heads were decondensed in a 25mM solution of DTT/2xSSC (DDT – commercially available solution of dithiothreitol, Sigma; SCC – 0.015M natrium citrate) for 3 minutes in 37°C, rinsed in 2xSCC for 30 minutes in 37°C and, finally, dehydrated in a series of increasing concentrations of alcohol solutions (70%, 80%, 95%).

We used the commercially available MultiVysion PGT test, which includes probes specific for chromosomes 13 (13q14), 18 (D18Z), 21 (21q22.13), X(DXZ1) and Y(DYZ3). Each probe was directly labeled with fluorochrome – SpectrumRed, SpectrumAqua, SpectrumGreen, SpectrumBlue and SpectrumGold, respectively. All the stages of FISH assessment were performed in exact accordance with the manufacturer's instructions. The cytogenetic samples were assessed with the Nikon Microphot FXA microscope with monostriatic optical filters – FITC (b-2A; Nikon), TRITC (G-2A; Nikon), Blue (VYSIS), Aqua (VYSIS), Yellow (VYSIS). The microscopic scans were registered by a monochromatic, cooled CCD Photometrics camera (Photometrics Quantix, KAF 1400) and analyzed with Multi-Scan software for image analysis (Computer Scanning System).

This particular choice of probes arose from the fact that only aneuploidies of spermatozoal chromosomes 13, 18, 21, X and Y may present in live-born children; being observed in 0.31% of neonates [15]. The trisomies and monosomies of all other chromosomes are lethal during embryogenesis.

Statistical methods

The results were analyzed with Student's t-test (CSS-Statistica 5.0). Values are given as mean ± standard deviation; minimum-maximum.

Results

Altogether we assessed 92800 spermatozoa obtained from patients before chemotherapy, 48500 spermatozoa obtained after chemotherapy and 61331 spermatozoa obtained from the control group. The mean number of spermatozoa analyzed in a single seminal sample was approximately 3000. However, 3 patients after chemotherapy failed to produce such a number of spermatozoa per sample due to profound oligozoospermia.

The chosen method of decondensation allowed to maintain optimal morphology of spermatozoal heads and preserved the structures of spermatozoal tails. Decondensation also provided significant background reduction, which, in turn, allows for a clearer interpretation of the results, especially in view of the simultaneous use of five probes.

In case of both the control group and the patient group before chemotherapy the efficacy of hybridization exceeded 90%. On the other hand, in the post-chemotherapy group it was significantly lower, not exceeding 60%.

Analysis of the number of obtained hybridization signals read by the probes allowed to determine the presence of aberrations in the number of copies of the investigated chromosomes. Two signals signified disomy. In such spermatozoa the overall number of chromosomes was increased (they were hyperhaploidal; i.e. >1n). Lack of signal was a sign of nullsomy (lack of chromosomal copy; i.e. < 1n – hypohaploidal spermatozoa). If doubled signals were discerned on all the probes simultaneously they signified the presence of a double chromosme set within the cell, i.e. diploidy (2n).

On applying the FISH technique with chromosomal centromer probes for chromosomes 13, 18, 21, X and Yto the spermatozoal samples of both the patients and the controls the following types of cells were identified:

(i) normal haploidal spermatozoa giving off a single hybridization signal from all the analyzed chromosomes – a feature characteristic for monosomy,
(ii) aneuploidal spermatozoa with no hybridization signal for one of the analyzed chromosomes – a feature characteristic for nullsomia,
(iii) diploidal spermatozoa releasing two hybridization signals for each of the analyzed chromosomes.

The percentages of the different types of spermatozoa acc. to the number of chromosomes are presented in Table II. No statistically significant differences were found between the groups.

The percentages of aneuploidal spermatozoa are compared in Table III. No statistically significant differences were found between the groups.

Table IV presents the populations of hyperhaploidal spermatozoa – i.e. those with autosomal disomy. No statistically significant differences were found between the groups.

Table V presents the populations of hypohaploidal cells i.e. without one of the analyzed chromosomes. No statistically significant differences were found between the groups.

Table VI presents the populations of spermatozoa according the number of X and Y chromosomes. The differences do not reach statistical significance. The mean percentages of spermatozoa containing both the X and Y chromosomes were significantly higher ($p<0.05$) than the percentages of XX and YY spermatozoa. All the other differences did not reach statistical significance.

Spermatozoa nullsomic as to the XY chromosomes constituted 0.14% in the control group, 0.19% in the group of patients before chemotherapy and 0.13% in the

Table II. Differentiation of the spermatozoal population within the seminal sample acc. to the number of chromosomes

Group (no. of pts. analyzed)	Spermatozoa with haploidal karyotype (%)	Spermatozoa with an anueuploidal no. of chromosomes (%)			
		Spermatozoa with hypohaploidal karyotype <1n (%)	Spermatozoa with hyperhaploidal karyotype >1n (%)	Diploidal spermatozoa (%) 2n	Aneuploidal spermatozoa summed (%)
Controls (5)	98.24	0.40	0.73	0.63	1.76
Pts. before chth (20)	98.0	0.40	0.91	0.69	2.00
Pts. after chth (20)	97.91	0.45	0.97	0.67	2.09

1n – 23 chromosomes / cell >1n – >23 chromosomes / cell
<1n – < 23 chromosomes / cell 2n – 46 chromosomes / cell

Table III. Differentiation of aneuploidal cells in the examined seminal samples

Group (no. of pts. analyzed)	Spermatozoa with aneuploidal karyotype 100%		
	Hypohaploidal spermatozoa <1n (%)	Hyperhaploidal spermatozoa >1n (%)	Diploidal spermatozoa 2n (%)
Control (5)	22.70	41.50	35.80
Pts. before chth (20)	25.98	42.12	31.90
Pts. after chth (20)	20.40	47.10	32.50

1n – 23 choromosomes / cell >1n – >23 chromosomes / cell
< 1n – <23 chormosomes / cell 2n – 46 chromosomes / cell

Table IV. Hyperhaploidal spermatozoa ratio (mean ± standard deviation)

Group (no. of pts. analyzed)	Autosomes (%)			
	13	18	21	Total
Control (5)	0.23±0.25	0.20±0.31	0.21±0.07	0.64
Before chth (20)	0.25±0.35	0.24±0.31	0.25±0.24	0.74
After chth (20)	0.31±0.40	0.23±0.35	0.29±0.20	0.83

Table V. Hypohaploidal nullosomic cells (mean ± standard deviation)

Group (no. of pts. analyzed)	Chromosomal nullosomies %			
	13	18	21	X/Y
Control (5)	0.13±0.09	0.09±0.12	0.07±0.05	0.14±0.14
Before chth (20)	0.08±0.15	0.08±0.08	0.08±0.06	0.19±0.19
After chth (20)	0.09±0.24	0.04±0.17	0.04±0.07	0.13±0.13

Table VI. Results of analysis of number of copies of sex chromosomes X/Y (mean ± standard deviation)

Group (no. of pts. analyzed)	Normal spermatozoa		Disomies			Nullsomies
	X (%)	Y (%)	XX (%)	XY (%)	YY (%)	X/Y (%)
Control (5)	48.24±3.40	51.76±3.40	0.075±0.10	0.50±0.28	0.14±0.15	0.14±0.14
Before chth (20)	45.8±3.40	54.2±3.40	0.19±0.10	0.49±0.40	0.12x0.12	0.19±0.19
After chth (20)	47.6±2.9	52.4±2.9	0.15x0.10	0.50x0.50	0.15±0.15	0.13±0.13

same group after chemotherapy. These values also failed to reach statistical significance.

Table VII presents the different forms of spermatozoa with diploid (2n) sets of chromosomes comparing them to the XY chromosomes in the analyzed semen samples. In the control group diploid karyotype XX spermatozoa constituted 0.05%, XY – 0.49% and YY – 0.09%. In the patient group these values reached 0.09%, 0,5% and 0.07% before chemotherapy and 0.09%, 0.49% and 0.07% after chemotherapy. The differences between the three groups did not reach statistical significance; diploidal XY spermatozoa were more common ($p<0.05$) than the XX and YY spermatozoa in all the three groups.

Spermatozoa with disomy of sex chromosome were much more common ($p<0.05$) than spermatozoa with autosomal disomy (13, 18 and 21). Such differences were observed in all the three groups, which suggests

Tabela VII. Differentiation of diploidal spermatozoa in relation to sex chromosome status in the examined seminal samples (mean ± standard deviation)

Group (no. of pts. analyzed)	Diploidal XX chromosome spermatozoa (%)	Diploidal XY chromosome spermatozoa (%)	Diploidal YY chromosome spermatozoa (%)
Control (5)	0.05±0.09	0.49±0.27	0.09±0.15
Before chth (20)	0.09±0.11	0.50±0.34	0.07±0.08
After chth (20)	0.09±0.06	0.49±0.26	0.07±0.12

a significantly higher incidence of disomy of the sex chromosomes.

Discussion

Our knowledge concerning the cytogenetic disorders of spermatozoa after chemotherapy remains incomplete. Some congenital diseases (such as Down syndrome or Patau syndrome) arise from evident genetic disorders of reproductive cells, although we are not aware of the reasons for their appearance nor of their relationship with parental exposure to noxious stimuli. Only a few papers concerning this subject have been published to date, and they contain contradictory data. In all these reports very few cases are reported (4-5 pts) [15-19]. Data concerning the influence of chemotherapy on the frequency of spermatozoal chromosome aberrations (evaluated with FISH and acc. to Yanagimachi modified by Brandiff) is also contradictory. Genesca and other authors have reported a significant increase of numerical and structural aberrations of spermatozoal chromosomes observed during the first 2-7 years after the completion of chemotherapy [16, 18]. On the other hand the study of Martin conducted on a group of patients between 2 and 13 years after the completion of chemotherapy [19] did not confirm the former findings.

The FISH technique (fluorescent *in situ* hybridization) has been used for over a decade to evaluate the frequency of chromosomal aneuploidy in the male reproductive cells of sperm donors, patients with gonadal tumours and carriers of balanced structural aberrations. Similar analyzes are also performed in men, whose offspring present with aneuploidy of chromosomes 21, X and Y in order to assess the risk of genetic defects in other children. Attempts have been made to analyze the connection between the age of the father and the risk of producing a child with a defective fenotype arising from chromosomal aneuploidy. The FISH technique has also been used to assess chromosomal structural aberrations in spermatozoa [20, 21]. There also exist pioneer studies in which hybridization was performed with the painting probe specific for one chromosome only [21]. However, such an approach does not allow to discern between disomic and diploidal spermatozoa and between nullsomic and non-informative (non-hybridized) spermatozoa. Attempts have been made to discern diploidal and disomic spermatozoa according to the size of the head. However, the correlation between the size of the head and the diploidal karyotype does not appear convincing, all the more so because in the course of decondensation the spermatozoal head increases [20]. The use of FISH with two molecular probes allows for a more exact evaluation of aneuploidy of spermatozoal chromosomes. The probability of simultaneous disomy of both the analyzed chromosomes (two hybridization signals) is so low, that a reading of double disomy may be interpreted as diploidy. This allowed to discern between diploidy and disomy, and also enabled us to evaluate the efficacy of the method. Concomittant use of three probes – two homologous for the X and Y chromosomes and one autosomal chromosome has allowed to evaluate the ploidy of the chromosomes [20].

The application of FISH with the simultaneous use of several molecular probes allows for a precise evaluation of the frequency of chromosomal ploidy in human spermatozoa [22]. When conducted in healthy, fertile men such studies enable us to assess the frequency of aneuploidy in each and every one chromosome of the human genome [22]. In his study Bernardini has shown that the frequency of aneuploidy of the studied spermatozoal chromosomes varies among the subjects, remaining between 0.01% and 0.4% [22]. These variations are probably caused by differences brought on by the method or by the criteria applied during specimen analysis in the different laboratories. They may also arise from individual variations between sperm donors.

In the studies concerning the frequency of chromosomal aberrations in the spermatozoa of patients with testicular cancer after orchidectomy alone this parameter showed no increase, as compared to healthy men in comparable age groups [15, 23].

Recent studies have been aimed at the influence of chemotherapy on the frequency of aneuploidy of chosen chromosomes [16, 18, 19]. Reports on the impact of chemotherapy on the frequency of aneuploidy of spermatozoal chromosomes present differing results.The FISH technique with the use of probes specific for chromosomes 1, 12, X and Y has been used to analyze spermatozoa obtained from patients after chemotherapy with the MACOP-B regime (methotrexate, leucovorin, doxorubicin, cyklophosphamide, vincristine, prednisone and bleomycin) [24]. In this material the frequency of spermatozoa with disomy did not differ from their frequency in the control group, reaching 0.1% for chromosome 1, 0.11% for chromosome 12, 0.05% for chromosome XX, 0.05% for chromosome YY and 0.18% for chromosome XY.

Similar studies were conducted in testicular cancer patients before and after chemotherapy with platin preparations. The results show a similar frequency of disomy of all the analyzed chromosomes – i.e. 0.11% and 0.06% respectively for chromosome 1; 0.18% and 0.15% for chromosome 12; 0.1% and 0.9% for chromosome XX; 0.13% and 0.1% for chromosome YY and 0.25% and 0.2% for chromosome XY [23]. However, there exist reports of a significant increase of the frequency of XY disomy in testicular cancer patients before (0.33%) and after (0.34%) chemotherapy acc. to the BEP regime, as compared to its frequency before treatmen – i.e. 0.14% [25]. A significant, though transient, increase in the hyperhaploidy of chromosomes 1, 12, X and Y has also been observed in men with Hodgkin's disease who had undergone NOVP treatment (novantrone, vincristine, vinblastine and prednisone). The percentages of aneuploidal spermatozoa decreased to pre-treatment values 100 days after chemotherapy application.

The present study was the first in which simultaneous hybridization with five probes (specific for chromosomes 13, 18, 21 X and Y) was performed in spermatozoal specimens. These particular chromosomes were chosen because only their numerical aberrations are discerned postnataly. The basic profit from this diagnostic approach is the possibility of evaluating the frequency of ploidy of five pairs of homologous chromosomes during a single analysis and in a relatively short period of time. Conducting such a complex analysis in one sperm sample is all the more important in subjects in whom it is difficult to obtain an adequate amount of semen and in subjects with profound oligozoospermia.Our results have not revealed any statistically significant differences between testicular cancer patients before and after chemotherapy and between these two groups and healthy controls regarding the frequency of aneuploidy of chromosomes 13, 18, 21, X and Y.

The statistically insignificant differences in the ratio of aneuploidal spermatozoa which we have observed may be caused either by individual variations or may depend on the age of the patients. The mean percentage of XY disomy spermatozoa were significantly ($p<0.05$) higher than the percentages of XX and YY disomy spermatozoa. Also disomy of sex chromosomes was more common than disomy of autosomal chromosomes (13, 18 and 21). Errors occurring during the first meiotic division are usually responsible for the lack of division of mother-derived chromosomes, while errors occurring during the second meiotic division are responsible for the lack of division of father-derived chromosomes. Additionally all errors of the first meiotic division (both mother- and father-derived) may cause a decrease in the frequency of recombinations, and thus predispose to the lack of chromosome division due to the loss of heterozygocity. The lack of recombination within the PARX and PARY has been described in literature. It a majority of cases it presents as 47,XXY. A similar decreased frequency of recombinations may observed in the case of pairs of acrocentric chromosomes [27]. The highly frequent appearance of XY spermatozoa (lack of division of chromosomes during the first meiotic division)as compared to XX and YY spermatozoa (lack of division of chromosomes during the second meiotic division) nay suggest that errors are more common during the first meiotic division. However the results are not easy to interpret, especially because similar observations have also been made in the control group. This may suggest that the above-mentioned phenomenon may not be an effect of treatment but, rather, arises from the variations ion the patient's age (over 25 years).

It is also interesting to note that we have observed a relatively low percentage of X and Y nullsomic spermataozoa (0.028% – 0.05%) although in 80% of cases of X monosomy the lost X chromosome is father-derived. It may suggest that monosomy of the mother-derived X chromosome may be caused by postzygotic mitotic errors, which cause the loss of the father-derived X chromosome. It may be connected with some form of father-dependant genomic imprinting which may affect some yet unidentified sequences localized within the X chromosome, which in turn causes a tendency to lose the father-derived X chromosome.

Our results show testicular cancer patients have a low risk of producing a child with chromosome 13, 18, 21, X or Y aneuploidy 30 months after the administration of platin oncolytics. One cannot rule out the possibility that platin oncolytics do cause transient chromosomal aneuploidy in the analyzed group of patients. However, all patients were studied 30 months after the completion of oncolytic treatment and therefore such a transient effect may have been lost from observation. On presenting these patients with a medical opinion one cannot forget about all other factors responsible for generating aneuploidal karyotypes. According to present day knowledge these patients should be referred to Genetic Clinics and, possibly, advised to perform pre-natal diagnostics.

Evaluation of the frequency of spermatozoal chromosomal aberrations is, at present, of crucial importance in sperm donors-carriers of balanced chromosomal aberrations (translocations, insertions and marker chromosomes) and in cancer patients in whom chemotherapy and/or radiotherapy are scheduled. Multicoloured FISH technique may be helpful in defining the risk of aneuploidy of chromosomes 13, 18, 21, X and Y in these two groups, especially in subjects with profound oligozoospermia. When accompanied by other diagnostic tools the FISH technique may allow to evaluate the important prognostic factors affecting the choice of the optimal treatment methods.

The ratio of spermatozoa containing the X chromosome as compared to spermatozoa containing the Y chromosome reached 48.24% and 51.76% in the control group; 45.80% and 54.20% in patients before chemotherapy and 47.60% and 52.40% in patients after chemotherapy. In a majority of cases we observed a slight increase in the percentage of Y-chromosome containing spermatozoa. However the differences in the ratios of X chromosome spermatozoa and Y chromosome sperma-

tozoa did not achieve statistical significance in any of the three groups. Thus we have found no statistically significant variation of the 50%: 50% X:Y ratio. Our results remain in concordance with the results published by other authors [17, 24].

Conclusions

Aneuploidy of chromosomes 13, 18, 21, X and Y is as common among healthy men as in a group of testicular cancer patients.

The use of platin-derived oncolytics does not affect the frequency of chromosome 13, 18, 21, X and Y aneupliody in testicular cancer patients 30 months after the completion of therapy.

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Paper received: 12 June 2003
Accepted: 10 July 2003