Carcinoma in situ of the testis: aneuploid cells in semen

ALEKSANDER GIWERCMAN, OLE PETTER F CLAUSEN, NIELS E SKAKKEBÆK

Abstract

The content of cellular DNA in ejaculates from eight patients with carcinoma in situ of the testis and 26 controls without evidence of testicular neoplasia was studied by flow cytometry. An aneuploid cell population with a ploidy value similar to that found for carcinoma in situ cells was detected in seminal fluid from four of the eight men with carcinoma in situ but in none of the controls. One year after orchidectomy or local irradiation in these four men no aneuploid cells were found in the semen.

These findings show that a detectable proportion of malignant germ cells may be released into the seminal fluid of patients with carcinoma in situ of the testis. Analysis of seminal fluid may therefore aid in screening for early neoplasia of the testis.

Introduction

Carcinoma in situ of the testis is an abnormal germ cell pattern of the seminiferous epithelium. The condition is asymptomatic and precedes invasive germ cell cancer.¹ If the neoplasia is detected at the stage of carcinoma in situ the patient can be cured by local irradiation or orchidectomy without further treatment.^{2,3} To date surgical biopsy has been the only means of diagnosing carcinoma in situ, there being no non-invasive diagnostic method available.

Spermatogonia as well as spermatocytes and spermatids have been found in semen,^{4,5} and recently the presence of morphologically abnormal cells in seminal fluid of men with testicular cancer has been reported.⁶ Though carcinoma in situ germ cells are also constituents of the seminiferous epithelium, they have not been detected in the ejaculate. Germ cells exfoliated from seminiferous epithelium are often difficult to identify morphologically in semen.⁴ Carcinoma in situ germ cells, however, have special biochemical and biological characteristics including an aneuploid DNA content,⁷ which is considered to be a marker of neoplasia.⁸

DNA flow cytometry is a sensitive and practical method of classifying isolated germ cells according to DNA content.⁹ Furthermore, with this method cells containing haploid, diploid, and tetraploid DNA have been detected in semen samples from barren couples.⁵ We have used DNA flow cytometry to examine semen for aneuploid cells in controls and patients with carcinoma in situ of the testis in an attempt to establish a non-invasive method for detecting carcinoma in situ.

Patients and methods

We studied semen samples from eight patients with carcinoma in situ of the testis (age range 24-41; table I) and from 26 controls (aged 21-36). The diagnosis of carcinoma in situ was established by testicular biopsy. In five

patients the biopsy was performed because they had a unilateral malignant tumour of the testis. The remaining three patients participated in screening for carcinoma in situ of the testis among men with a history of cryptorchidism. Routine semen analysis showed a median sperm density of 0.8 (range 0.19) $\times 10^9/l$ in patients with carcinoma in situ and 14 (range 0.100) $\times 10^9/l$ in controls (normal $>20 \times 10^9/l$). All men with carcinoma in situ delivered a pretreatment ejaculate, and from four of the patients semen samples were also collected one year after treatment by orchidectomy or local irradiation (see tables I and II).

TABLE I—Clinical and laboratory data on patients with carcinoma in situ of testis

Case No	Age (years)	Histological findings		Treatment
		Right testis	Left testis	
1	25	Previously removed for cancer	Carcinoma in situ	Local irradiation
2	25	Carcinoma in situ	Reduced spermatogenesis	Orchidectomy
3	37	Previously removed for cancer	Carcinoma in situ	Local irradiation
4	41	Previously removed for cancer	Carcinoma in situ	Local irradiation
5	24	Carcinoma in situ	Normal spermatogenesis	Orchidectomy
6	26	Carcinoma in situ	Normal spermatogenesis	Orchidectomy
7	26	Carcinoma in situ	Previously removed for cancer	Local irradiation
8	25	Carcinoma in situ	Previously removed for cancer	Local irradiation

TABLE II—Results of seminal analysis

Case No	Pretreatment sperm density ($\times 10^9/l$)	Cellular DNA content*					
		Before treatment			One year after treatment		
		2c	4c	Aneuploidy	2c	4c	Aneuploidy
1	0	+	+	+	+	—	—
2	2.8	+	+	+	+	—	—
3	0	+	+	+	+	+	—
4	0	+	+	+	+	+	—
5	19.0	+	+	—	Not analysed		
6	Not analysed	+	—	—	Not analysed		
7	1.6	+	+	—	Not analysed		
8	Not analysed	+	+	—	Not analysed		

*2c=Diploid peak. 4c=Tetraploid peak.
+/- = Present/absent.

Controls were seven healthy men, one patient with epididymitis, 13 men attending an infertility clinic, four men with bilateral cryptorchidism, and one man who 14 months earlier had had a testicle removed for unilateral cancer. Testicular biopsy in the four men with cryptorchidism and the man with unilateral orchidectomy showed no changes of carcinoma in situ. Testicular biopsy was not done in the remaining 21 controls, who had no signs of testicular neoplasia. The risk of having carcinoma in situ of the testis was below 1% for each of these controls.¹⁰ All controls delivered one semen sample.

After liquefaction, and within two hours, aliquots of semen samples were washed once in 0.9% sodium chloride and centrifuged in 1M sucrose at 20 000 g. The pellet was resuspended in 2 ml 0.9% sodium chloride, fixed in 96% ethanol, and stored at 4°C before preparation for flow cytometry.

DNA flow cytometry—After storage for several days the samples were prepared for DNA flow cytometry as described.⁹ Briefly, the cells were washed in 0.9% sodium chloride, incubated at 37°C for one hour with ribonuclease, then incubated with 0.4% pepsin at 37°C for 15 minutes before staining with ethidium bromide. The resulting DNA fluorescence was stable for about 24 hours when the samples were kept on ice. DNA fluorescence was measured with an Ortho cytofluorograph 50H connected to a 2150 computer (Westwood, Massachusetts). The instrument was equipped with an argon ion laser and the cells excited with 200 mW at a wavelength of 488 nm. Lymphocytes were used as diploid DNA standard and always prepared together with the ejaculates. After having been measured without

Laboratory of Reproductive Biology, University Department of Obstetrics and Gynaecology, Rigshospitalet, Copenhagen, Denmark

ALEKSANDER GIWERCMAN, MD, research fellow

University Department of Pathology, Rikshospitalet, Oslo, Norway

OLE PETTER F CLAUSEN, MD, DR MED, professor of pathology

Laboratory of Reproductive Biology, Rigshospitalet, and University Department of Paediatrics, Hvidovre Hospital, Copenhagen, Denmark

NIELS E SKAKKEBÆK, MD, DR MED, professor of paediatrics

Correspondence to: Dr A Giwerzman, Laboratory of Reproductive Biology, Rigshospitalet 4052, Blegdamsvej 9, Copenhagen, Denmark 2100.

internal standard lymphocytes were added to each semen sample. All DNA fluorescence histograms were evaluated blindly by the same investigator, who did not know the origins of individual semen samples.

Results

A peak in the diploid region was seen in almost all oligospermic samples and in some samples with normospermia. In some cases a diploid peak was associated with some cells in the tetraploid region. In addition to some diploid cells, a distinct aneuploid cell population was detected in pretreatment semen samples from four of the eight patients with carcinoma in situ of the testis (table II) but not in the ejaculate from any of the 26 controls (fig 1). One year after treatment for carcinoma in situ of the testis

FIG 1—DNA fluorescence of cells in pretreatment ejaculates from cases 2 and 4 and a control. Peaks in diploid (2c) regions may represent Sertoli cells, spermatogonia, cells from lining epithelium of excretory ducts, or (occasionally) inflammatory cells. Pattern in case 2 was similar to that in case 1. Diploid cells were present in all ejaculates and aneuploid cells detected in pretreatment ejaculates of two patients with carcinoma in situ of testis (peaks to right of diploid peak). Two aneuploid DNA stemlines were detected in ejaculate from case 4. Analysis did not allow optimal categorisation of sperm fluorescence, represented by pulses to left. (4c = Tetraploid region.)

aneuploid cells were no longer detectable in ejaculates from any of the four patients (fig 2; table II). In one of the four pretreatment samples two aneuploid DNA stemlines were detected (fig 1; case 4). In three samples with aneuploid cells there were fewer diploid cells than aneuploid cells; in the fourth sample the numbers were almost equal. Over 2000 aneuploid cells were identified in each of the positive cases. Based on these measurements we assume that several hundred aneuploid cells must be present to allow detection.

The median ploidy value of the aneuploid peaks was 2.8c (range 2.7-3.6c), 2c corresponding to the diploid DNA content.

Discussion

In this study aneuploid cells were detected in semen from four of the eight men with carcinoma in situ of the testis. Though the aneuploid cells were not identified morphologically, we have several reasons to believe that they were carcinoma in situ cells that had been exfoliated from the testis.

Firstly, the median ploidy value of 2.8c found for the abnormal cell population in the semen of these patients was almost identical with that found by Müller *et al* in a microspectrophotometric study of carcinoma in situ cells in testicular tissue.⁷ Secondly, aneuploid cells were not detected in semen samples obtained one year after treatment from the four patients with pretreatment semen aneuploidy. In a pretreatment biopsy sample of the remaining testis in case 1 all seminiferous tubules contained exclusively carcinoma in situ and Sertoli cells. As only Sertoli cells were found in a follow up

FIG 2—Case 3. DNA fluorescence of cells in ejaculates before and one year after treatment of carcinoma in situ of testis. Peak between diploid (2c) and tetraploid (4c) regions before treatment represents aneuploid cells. Aneuploid cells disappeared from ejaculate after treatment. Pulses to left of diploid peak due to sperm fluorescence (for more details see fig 1).

biopsy sample after irradiation, the disappearance of aneuploid cells from the ejaculate was most probably due to eradication of carcinoma in situ cells from the tubules. Lastly, we do not believe that the aneuploidy found in the semen of the four men with carcinoma in situ was due to emission of non-malignant germ cells, as no aneuploid cell fraction was detected in the controls, who included men with normal and impaired spermatogenesis. Neither was aneuploidy found in previous flow cytometric studies of semen samples from men attending an infertility clinic,⁸ nor in testicular fine needle aspirates from normal and infertile men.¹¹ Studies of cell morphology and cell markers in semen from patients with carcinoma in situ of the testis may contribute to the final identification of the aneuploid cells as malignant germ cells.

Development of a reliable method for detecting carcinoma in situ of the testis by seminal analysis will have important clinical implications, as it will facilitate screening for testicular carcinoma in situ and thus aid in preventing invasive testicular cancer. That no aneuploidy was detected in the semen of four men with carcinoma in situ may mean that the technique needs further refinement to improve sensitivity or that no or very few aneuploid cells were

present in these ejaculates. This last may have been due to obstruction of excretory ducts or to only a very few tubules having changes of carcinoma in situ, so releasing too few cells to be detected.

Higher sensitivity in identifying patients with carcinoma in situ may be achieved by immunocytochemical marker studies in seminal cells. Placental-like alkaline phosphatase has been reported as a marker of carcinoma in situ cells of the testis.^{12,13} A recently developed monoclonal antibody—M2A—seems to be a more sensitive marker for detecting these malignant germ cells.¹⁴

In conclusion, our study shows that semen from a proportion of men with carcinoma in situ of the testis contains cells with aneuploidy similar to that of carcinoma in situ germ cells of the testis. Analysis of seminal fluid may therefore aid in the early detection of testicular neoplasia in screening for carcinoma in situ of the testis and in the follow up of patients treated for carcinoma in situ of the testis.

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ONE HUNDRED YEARS AGO

MUCH has been said and written during the last year or two regarding the development of the health resorts of this country, and at this season it seems opportune to inquire what may fairly be aimed at in this direction, and what is clearly ill-advised and chimerical. It can hardly be denied that in the art of popularising sanatoria and rendering them thoroughly attractive, we have still much to learn from our Continental neighbours. Some of the most noted resorts in Europe have few natural advantages either of scenery, position, or resources, but in many cases art has been admirably utilised to supplement Nature. The gay Kursaal, with its daily round of amusements, the pleasant parks and trim gardens, the admirably appointed *établissements des bains*, the excellent music—all these are but parts of the ordinary stock in trade with which Wiesbaden and Carlsbad, Homburg and Vichy so successfully attract their crowds of annual patrons. But of such attractions our British resorts are for the most part singularly destitute. They may have beautiful scenery, valuable mineral waters, excellent air, but these important advantages do not save them from being in many cases intolerably dull. Our error is twofold: we make the theoretical mistake of regarding health resorts as places solely for definite medical treatment, forgetting that amusement and mental distraction are not only pleasurable to all, but absolutely indispensable to the sick; and we fall into the practical error of assuming that sanatoria can provide sufficient recreation without organisation and co-operation. We need to realise, first, that a health resort has not fulfilled all its duty when it has built fine hotels, secured an impeccable water-supply, and cleansed its drains; and, secondly, that adequate distraction and amusement cannot safely be left to the accidents of individual enterprise, but require united and municipal effort. A health resort must first be healthy, and secondly attractive. It must aim at securing as many as possible of the conditions which check disease and promote recovery, and it must provide ample and varied relaxation for convalescents and their companions. Nor should it be forgotten that the presence of every invalid entails on an average the presence of two or three others, friends or attendants, who are not invalids. A health resort which provides sufficient occupation and amusement for this latter class will have gained an additional claim to consideration.

While we are still far behind France and Germany in enterprise of this kind, it would be unjust to ignore the great progress which is being made. Southport shows what a pleasant resort can be constructed upon the barest of sandhills, and Bournemouth is a witness of how a pine forest can be transformed into one of the most attractive of sanatoria. Bath, Leamington, and Droitwich are proving that enterprise is not hopelessly dead at the English spas and here, as always, well-directed effort is reaping its due reward. No British resort has hitherto, so far as we are aware, adopted the plan so general on the Continent of imposing a fixed tax upon all visitors for the development of the locality on the lines indicated in this article. Such an impost would be a novelty in this country, and perhaps somewhat repugnant to our prejudices, but a nation that still tolerates tolls upon bridges cannot consistently rebel against a *Kur-tax*. The advantages of such an arrangement need hardly be pointed out.

After all has been said, it may be urged that continental resorts surpass us in point of climate, and that as long as our summers and autumns are prone to be sunless and damp, so long will those who have the means migrate to more favoured regions. Granting this argument some weight, it by no means disposes of the entire question. There can be little doubt that at the holiday season our climate is far more promotive of health than that of many of the most famous sanatoria. From July to October includes the period at which our various classes seek their annual holiday. During July and August sea-bathing is one of the chief ends pursued; and it may be confidently asserted that no marine resorts in the world equal those of Great Britain. Scarborough, Hastings, Brighton, Eastbourne, Ilfracombe, Cromer, Whitby, Llandudno, Tenby, and Barmouth constitute a list that might easily be enlarged, and one to which no Continental country can afford an adequate parallel. Another large class of holiday-seekers is composed of those who require mineral waters and baths, which are usually associated. Now, with one important exception, this country is well provided with natural mineral springs, and it can hardly be denied that in summer or early autumn Harrogate, Buxton, and Moffat are more desirable resorts than the German or Austrian baths. Unfortunately, our springs are deficient in waters aerated with carbonic acid gas, and hence our sulphur and saline waters are apt to prove somewhat heavy and indigestible. It has been proposed, and in places successfully attempted, to have some species of natural water artificially aerated.

But, to recur to our original position, it is not, we think, any natural deficiency in our health resorts which dooms many of them to insignificance, so much as lack of enterprise and blindness to those attractive accessories which make the fortune of many of the Continental sanatoria. It must be owned that as a nation we are not successful in organising those minor enjoyments and lighter graces which are yet indispensable to a well-rounded existence. We "take our pleasures sadly," and thereby are great losers. Probably that incapacity for small enjoyments, that inability to be easily amused which so strongly characterises us, is a lingering relic of the old Puritan prejudice against every species of pleasure. Such a prejudice is happily almost a thing of the past. From the medical point of view it was an error to be deplored, and such errors, even when ostensibly abandoned, are apt to leave traces behind in the poise and adjustment of the national mind. We must not be ashamed to amuse the sick, and both sick and healthy may with advantage occasionally devote some serious thoughts to those arts which promote the best and most advantageous utilisation of leisure hours and holiday seasons. It is only a crude philosophy which can condemn such efforts as unworthy of our best endeavours and most serious attention.

This JOURNAL has taken many opportunities of pressing these views upon the attention of those most interested in their success, and the subject is one to which Mr. Ernest Hart referred in some detail in his "Letters from Carlsbad." It is gratifying to us to know that some good results are likely to follow. At the beginning of another season we again invite attention to a subject of national as well as professional importance.

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