Attachment (2) to Fechheimer Hearing Exhibit 138

PERSISTENT TUMOR CELLS IN THE VAGINAL SMEAR DURING THE FIRST YEAR AFTER RADIATION THERAPY OF CARCINOMA OF THE UTERINE CERVIX*

PROGNOSTIC SIGNIFICANCE

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INVASIVE carcinoma of the uterine cervix remains a significant health problem in some communities; at the Radiotherapy Department of the I. González Martínez Oncologic Hospital in San Juan this type of tumor constitutes 20 per cent of the present therapy patient work load.

During the last 20 years radiation therapists have been able to develop safe and efficient techniques for the radiologic treatment of carcinoma of the uterine cervix.

The curability results obtained by radiation therapy in patients with carcinoma of the uterine cervix justify its classification as the treatment of choice for this condition;[1] yet, despite present radiotherapeutic achievements, we fail to cure a significant number of patients. At our Department these treatment failures presently range from 10 per cent in Stage I cases to 55 per cent in patients with Stage III lesions.

The need exists to detect irradiation failures early enough so as to be able to administer additional therapy with a better chance of success; this may be of the nature of a surgical procedure or further irradiation. Present clinical means for the detection of postirradiation treatment failures do not always permit the discovery of early and favorable tumor manifestations; at times, it is most difficult to distinguish recurrent or persistent tumor from postirradiation normal tissue changes. The use of biopsy material for the detection of postirradiation persistence presents certain limitations;[2,6] a slowly regressing tumor may be mistaken for a persistent lesion.

The present report relates to a study of the prognostic significance of persistent tumor cells in the vaginal cytology smear during the first year after irradiation for carcinoma of the uterine cervix.

MATERIAL AND METHODS

At the end of 1960 a project was started at the Radiotherapy and Cancer Division of the Puerto Rico Nuclear Center with the aim of determining the value of exfoliative cytology as a prognostic test in patients with carcinoma of the uterine cervix submitted to irradiation. Vaginal smears were taken before, during, and after irradiation. Initially two smears were taken per case (one by scraping the cervix and the other by pipette suctioning of the vaginal pool). In 1964 it was decided that one slide could include both samples and this practice persisted. The smears were processed by the Papanicolaou method for microscopic study for persistent tumor cells and for evidence of radiation effects in the normal epithelium.

For the present analysis we have utilized the cytologic diagnoses of persistent tumor cells in the smears taken 1, 2, 4, 6, and 12 months post irradiation. Besides the initial cytologic impression, all the slides were reviewed again during the last year and the pathologist has relied on his present criteria of what is a tumor cell in the vaginal

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smear after irradiation for carcinoma of the cervix uteri. Cytologic impressions were classified as positive or negative for tumor cells, suspicious for tumor cells, or unsatisfactory.

The patients in this study were mostly of low socio-economic level who were referred to the I. González Martínez Oncologic Hospital for radiation therapy of histopathologically proven carcinoma of the uterine cervix. At times, because of economic reasons, they had difficulty in transportation to the hospital. A total of 342 cases were included in the study with minimal post-treatment follow-up of 3 years. For various reasons, not all cases seen during the study period were included. A few patients were unable to provide a satisfactory cytologic smear, and some, because of economic or other personal reasons, failed to return for follow-up during the first year. The reasons for exclusion were: persistent unsatisfactory vaginal smears; no available vaginal smear in the first post-treatment year; and treatment interruption before an adequate dose was delivered. The excluded cases constitute approximately 30 per cent of the cervical cancer work load in the Department during the time of the study.

Survival and tumor-free status were determined at 3, 4, and 5 years after radiation therapy. Four per cent of the cases could not be traced at the 5-year mark because of change in address or other reasons.

Using follow-up information from the patients' charts, we correlated tumor-free survival with the presence of persistent tumor cells in the vaginal smear after irradiation, taking into consideration the stage of the disease and the treatment modality.

TREATMENT TECHNIQUES

Patients in this study received external irradiation by orthovoltage roentgen therapy (half value layer of 3 to 4 mm. Cu) or cobalt 60 teletherapy, followed by intracavitary therapy. Anterior and posterior pelvic fields were used; the width ranged from 16 to 18 cm. and the height from 12 to 14 cm., depending on the size of the pelvis.

In the first 2 years of the study, roentgen therapy cases were treated by means of sacrosciatic fields, in addition to the anterior and posterior ones. The external irradiation doses were calculated as exposures in the mid-pelvis and ranged from 4,000 r in 6 weeks with roentgen rays to 4,500 r in 6 weeks with cobalt 60. For curietherapy, we utilized radium or cobalt 60 sources in the vagina and the uterus; the doses averaged 4,000 r calculated at point A.

THE IDENTIFICATION OF TUMOR CELLS IN THE POSTIRRADIATION VAGINAL SMEAR

Satisfactory postirradiation vaginal smears may contain benign and tumor cells with or without irradiation effect. The identification of tumor cells without irradiation effect offers no difficulty to the pathologist. Tumor cells with irradiation changes may show: bizarre appearance, giant and multiple nucleoli, marked vacuolization, and increased cell size. Likewise, benign cells may show comparable irradiation changes: increased size of the cell and the nucleus, conspicuous vacuolization, multinucleation, and nuclear changes (pyknosis, karyorrhexis, wrinkling and multiple nucleoli). At times, it may be difficult to differentiate between dysplastic cells and malignant cells, when they show pronounced irradiation changes. Some of these cases are labelled "suspicious."

A number of smears are found unsatisfactory for diagnosis; this may be due to the presence of too many leukocytes, when a purulent discharge is present or to scanty material with practically no identifiable cells.

INCIDENCE OF SMEARS NEGATIVE FOR TUMOR CELLS DURING THE FIRST YEAR POSTIRRADIATION

The number of cases showing smears negative for tumor cells after irradiation, at various post-treatment intervals, is shown in Table 1. This table excludes cases

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TABLE I

PER CENT OF NEGATIVE SMEARS BY MONTHS AFTER THERAPY

Months after Therapy	No. with Satisfactory Smear	Per Cent Negative
1	248	74
2	265	86
4	252	89
6	216	90
12	184	90

which were lost to follow-up and patients with unsatisfactory smears. By the end of 1 month post treatment, 26 per cent of cases showed smears suspicious or positive for tumor cells; this number rapidly decreased to the 10 per cent level found 6 and 12 months post therapy. The number of unsatisfactory smears ranged from 63 at 2 months post therapy to 144 twelve months post irradiation.

TUMOR-FREE SURVIVAL OF THE PATIENTS UNDER STUDY

The tumor-free survival status of all patients in the study, correlated with stage of disease and treatment modality is shown in Table II. The number of Stage IV cases is too small for proper statistical evaluation; the 5-year tumor-free survival in this group was 86 per cent for cobalt 60 teletherapy cases and 92 per cent for roentgen therapy patients. Stage II cases had a 5-year tumor-free survival of 80 per cent with cobalt 60 teletherapy and 52 per cent with roentgen therapy. Stage III cases had a tumor-free 5-year survival of 47 per cent with cobalt 60 teletherapy and 21 per cent with roentgen therapy. Stage IV cases in this study had no tumor-free 5-year survivors.

The correlation between presence of a negative smear at different post-treatment months and tumor-free survival for all stages is shown in Table III. The chances of living with no evidence of disease at 3, 4, and 5 years, after a negative smear, are shown in this table. The 5-year tumor-free survival goes up from 43 per cent when the smear is negative 1 month after irradiation to 65 per cent when this condition is found 12 months post treatment. The presence of a negative smear does not appear to be of prognostic significance in the first 4 months post irradiation.

Table IV shows the correlation of the

TABLE II

SURVIVAL BY TREATMENT MODALITY AND STAGE

Treatment	Stage	At 3 Years		At 4 Years		At 5 Years	
		No. Eligible	% NED	No. Eligible	% NED	No. Eligible	% NED
Cobalt 60	All	173	66	102	65	49*	63
	I	29	90	13	85	7	86
	II	87	69	51	69	20	80
	III	49	53	32	53	19	47
	IV	8	25	6	33	3	0
Roentgen Rays	All	155	51	123	48	91†	44
	I	22	91	17	88	12	92
	II	60	58	46	61	35	57
	III	70	34	57	28	42	21
	IV	3	0	3	0	2	0

* Excluding 2 patients lost to follow-up.
† Excluding 4 patients lost to follow-up.
NED = no evidence of disease.

TABLE III

RELATIONSHIP OF NEGATIVE SMEAR AND CHANCE OF BEING FREE OF DISEASE
All Stages

Months after Therapy	At 3 Years		At 4 Years		At 5 Years	
	No. Negative	% NED	No. Negative	% NED	No. Negative	% NED
1	183	56	114	49	67	43
2	227	61	60	56	97	52
4	224	63	164	60	105	58
6	194	67	145	61	84	57
12	165	72	122	67	68	65

NED = no evidence of disease.

presence of a negative smear, with tumor free survival for Stage I and II cases together; Table V shows this relationship for Stage III and IV cases. From these tables it appears that a negative cytology report 4 to 12 months post irradiation is associated with a good prognosis in the early stages (73 to 82 per cent tumor-free 5-year survival); however, in Stage III and IV a negative report is not associated with a particularly good prognosis.

The correlation of the presence of a smear positive for tumor cells in the post irradiation period with tumor-free survival is shown in Table VI. The chance of having tumor-free 5-year survival is extremely low

TABLE IV

RELATIONSHIP OF NEGATIVE SMEAR AND CHANCE OF BEING FREE OF DISEASE
Stage I and II

Months after Therapy	At 3 Years		At 4 Years		At 5 Years	
	No. Negative	% NED	No. Negative	% NED	No. Negative	% NED
1	113	67	63	62	33	64
2	131	75	89	71	52	73
4	138	76	96	75	61	77
6	124	76	87	72	46	74
12	107	82	74	81	39	82

NED = no evidence of disease.

TABLE V

RELATIONSHIP OF NEGATIVE SMEAR AND CHANCE OF BEING FREE OF DISEASE
Stage III and IV

Months after Therapy	At 3 Years		At 4 Years		At 5 Years	
	No. Negative	% NED	No. Negative	% NED	No. Negative	% NED
1	70	37	51	33	34	24
2	94	41	71	37	45	29
4	86	43	68	40	44	33
6	70	51	58	45	38	37
12	58	53	48	46	39	41

NED = no evidence of disease.

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TABLE VI

RELATIONSHIP OF POSITIVE SMEAR AND CHANCE OF BEING FREE OF DISEASE

Months after Therapy	At 3 Years		At 4 Years		At 5 Years	
	No. Positive	% NED	No. Positive	% NED	No. Positive	% NED
1	49	65	44(1)*	61	40(2)*	65
2	29	45	23	48	21	47
4	18	39	8	38	5	(0)
6	11	36	7	14	5	(20)
12	10	30	5	(20)	3	(33)

* Number of lost cases excluded.
NED—no evidence of disease.

when a smear has been found to contain tumor cells 4 or more months after therapy; however, the number of cases in this portion of the study is too low to justify any valid conclusion.

DISCUSSION

The results of this study show that exfoliative cytology of the vagina, used during the first year after irradiation for carcinoma of the uterine cervix, is of some relative value in the management of these cases when a satisfactory smear is obtained. Unfortunately, the number of smears considered unsatisfactory by the clinician at the time the sample was taken or by the laboratory personnel was high; this was so in 80 cases at 1 month, 63 at 2 months, 76 at 4 months, 112 at 6 months, and 144 at 12 months. The reasons for an unsatisfactory smear may be: excessive inflammatory changes in the vaginal canal, dry mucosa with resulting scanty material in the sample, stenosis of upper vaginal canal, and gross bleeding when adhesions are broken during the examination.

Another limiting factor in the use of this technique relates to the location of the persistent or recurrent disease; a good number of the recurrences are in the parametria or the pelvic wall. An analysis of sites of active disease at the time of death in patients within 5 years after radiation therapy for carcinoma of the uterine cervix at the M. D. Anderson Hospital in Houston, Texas, revealed that in the groups of patients dying with tumor limited to the pelvis, more than 50 per cent had no local disease.[6] Talbert and co-workers[7] have reported that in their cases 20–30 per cent of the recurrences were in the parametria.

Some cases with gross tumor may not have a positive cytology; these false negatives may be as high as 9 per cent.[7] The presence of grossly suspicious tumor on pelvic examination calls for diagnostic studies. Therapy should not be instituted until persistent disease is proven; inflammatory or postirradiation changes may mimic cancer. In a group of 991 cases with the initial diagnosis of recurrent or persistent cervix cancer at Memorial Hospital in New York, 14 per cent did not have cancer.[8]

The persistence of tumor cells in the smear in the first months post therapy has no significance. This finding becomes important at 4 months or later. This experience is different from that reported by Koss[2] who states that the presence of cancerous cells for a period of 4 weeks following the completion of radiation treatment for cervical cancer carries an ominous prognosis. The time of disappearance of the tumor cells from the smear is related to the rate of regression of the tumor after radiation therapy. It is well known that some tumors have very long volume doubling times, and consequently would grow or disappear very slowly. Although by the end of the first postirradiation month 75 per cent of cases will have negative smears, some cases will have persistent tumor cells 6 or 12 months post therapy and never show further evidence of disease. The finding of malignant cells in the smear up to 1 year after successful radiation therapy has also been reported by Von Haam and Allery.[8]

Regardless of the above mentioned exceptions, when we find persistent tumor cells 4 months or longer after therapy, we repeat the smear and if the finding is confirmed we proceed with diagnostic investigations that may include dilatation and curettage and cervical biopsy. When histologic evidence of the disease is found, we proceed with the definitive treatment of the recurrence.

Stage I and II cases, in particular, may benefit from the use of vaginal cytology for persistent or recurrent tumors; this should favor the treatment of these tumor manifestations when a better chance of success exists.

SUMMARY

1. A study of the prognostic significance of persistent tumor cells in the vaginal smear during the first postirradiation year in 342 patients with carcinoma of the uterine cervix is presented.

2. The number of negative and positive smears at 1, 2, 4, 6, and 12 months post irradiation is analyzed and a correlation is made with the tumor-free survival at 3, 4, and 5 years.

3. Persistent tumor cells in the vaginal smear have no prognostic significance when present before the 4th month post irradiation.

4. The absence of tumor cells in the smear taken 4 months or later after therapy is associated with a relatively good prognosis, particularly in Stage I and II.

5. The presence of tumor cells 4 months or later after therapy is associated with a poor prognosis.

6. An occasional patient may have tumor cells present in the vaginal smear as long as 12 months after treatment and never show further evidence of disease.

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