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## Tetrazolium staining in cytology of cancer of the female genitalia

by

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### INTRODUCTION

On the basis of previous investigations Ku et al. <sup>(1)</sup> developed a simple cytological technique for the diagnosis of cervical cancer with which they obtained satisfactory results.

The purpose of this paper is to evaluate the diagnostic accuracy of the method in malignant tumors of the female genital organs.

### MATERIAL AND METHODS

The analysis comprises 10101 clinic patients, some of whom were hospitalized and others ambulatory <sup>(2)</sup>.

The test was performed by the following technique:

a) with the aid of a speculum the upper end of the vagina is visualized and washed several times under high pressure with 8 to 10 ml of the reagent described by Ku et al. The lavage is carried out with an ordinary glass cytology pipette provided with a rubber bulb;

b) the washings from the vaginal vault are collected in a centrifuge tube and kept in a thermostatic bath at 37 to 38 °C for one hour or at room temperature for at least two hours;

c) one drop of the reddish sediment which has formed spontaneously is then placed on a microscopic slide and a coverglass;

d) the sediment is examined microscopically and a search made for cells whose cytoplasm is filled with refragent granulation of a brilliant orange-red color.

If cells answering to this description, of strawberry-like appearance and at least three times bigger than a leukocyte are detected, the result of the cytochemical test must be regarded as positive for cancer.

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## RESULTS

In table 1 the diagnostic accuracy of the cytochemical (TTC) method is compared with that of cytology and that of colposcopy in 320 cases of *invasive carcinoma of the cervix*. An analysis of the results indicates that in these cases the cytochemical test gave a higher number of false negative results than either of the other two methods.

Table 1. *Diagnostic accuracy of the triphenyltetrazolium chloride (TTC) method compared with that of cytology and that of colposcopy in 320 cases of invasive carcinoma of the cervix.*

Results	TTC method		Cytology		Colposcopy*	
	No of cases	per 100	No of cases	per 100	No of cases	per 100
Positive	293	91.5	295	92.1	268	83.7
Doubtful	11	3.1	19	5.9	45	14.0
Negative	16	5.0	6	1.8	7	2.1

\* Colposcopically, cases were regarded as doubtful in which the picture was not clearly neoplastic but nevertheless suggested the advisability of guided biopsy

The false negative results occur in all stages of invasive carcinoma and are not related to the histological type of neoplastic growth.

Since the findings of false negative results obtained by various individual techniques (cytochemistry, cytology and colposcopy) usually did not coincide, we analysed the results to see which combination of two methods gave the lowest number of false negative readings. In our 320 cases there was not a single instance of a false negative result by a combination of the colposcopic and the cytochemical techniques. Only one false negative result (0,3%) was obtained with the combination of either colposcopy and cytology or cytology and the cytochemical method. These results indicate that the cytochemical method may be used successfully for the detection of cancer of the cervix when two diagnostic techniques are applied concurrently as a routine.

In table 2 the results obtained with the cytochemical method in 39 cases of *carcinoma in situ of the cervix* are compared with those obtained with the two other methods.

Table 2. *Diagnostic accuracy of the triphenyltetrazolium chloride (TTC) method in 39 cases of carcinoma in situ of the cervix uteri compared with the cytological and colposcopic methods.*

Results	TTC method		Cytology		Colposcopy*	
	No of cases	per 100	No of cases	per 100	No of cases	per 100
Positive	15	38.4	25	64.2	7	17.9
Doubtful	4	10.2	7	17.9	29	74.3
Negative	20	51.3	7	17.9	3	7.7

\* See note underneath table 1.

From the table it can be seen that the cytochemical method is not satisfactory in cervical carcinoma in situ. The test was repeated several times in 16 of the 20 cases in which it was negative, and it remained negative. The incidence of false negative results by a combination of two methods in carcinoma in situ was as follows: 2,6% (one in 39 cases) by colposcopy combined with cytology, 5,2% (two cases) by colposcopy combined with the cytochemical method, 7,7% (three cases) by cytology combined with the cytochemical method.

The aggregate of *false positive results* in specimens obtained by cervico-vaginal lavage was 440 out of 7939 cases (5,5%) in which there were no malignant lesions. The incidence of doubtful results in the same group of 7939 cases was 198 (2,4%). An attempt is made to analyse the causes of false positive results:

- a) granulation tissue on the vaginal vault, 60 cases;
- b) hyperplasia of the endometrium, 58 cases;
- c) cervical dystrophy (ectropion, ectopy, epidermization), 52 cases;
- d) cervical polyps, 52 cases;
- e) puerperium, 40 cases;
- f) vaginitis, 37 cases;
- g) endometrial atrophy, 19 cases;
- h) radiation induced cervico-vaginal dystrophy, 16 cases;
- i) endometrial polyps, 16 cases;
- l) fibromyoma of the uterus, 14 cases;
- m) pregnancy, 10 cases;
- n) cervical dysplasia, 6 cases;
- o) endometritis, 5 cases;
- p) endometriosis, 3 cases;
- q) genital prolapse, 2 cases;
- r) amenorrhoea, 1 case;
- s) adnexitis and/or pelvic peritonitis, 1 case;
- t) genital malformation, 1 case.

The false positive results occur most frequently in cases in which histiocytic elements of the macrophage type are present in the smear.

In table 3 the results obtained with the triphenyltetrazolium chloride (TTC) method are compared with those obtained by cytology in 122 cases of *carcinoma of the endometrium and/or of the upper portion of the cervical canal* (i.e. with no colposcopically demonstrable ectocervical signs).

Table 3. *Diagnostic accuracy of the triphenyltetrazolium chloride (TTC) method and of the cytology in 122 cases of carcinoma of the endometrium and/or of the cervical canal (upper portion).*

Results	Cytochemical examination (TTC)		Cytological examination	
	Cervicovaginal lavage	Endometrial lavage	Pap smear from pool	Endometrial aspiration
Positive	76 cases out of 122 (62.2%)	65 cases out of 68 (95.5%)	58 cases out of 122 (47.5%)	28 cases out of 35 (80%)
Doubtful	5 cases out of 122 (4%)	1 case out of 68 (1.4%)	35 cases out of 122 (28.6%)	4 cases out of 35 (11.4%)
Negative	41 cases out of 122 (33.6%)	2 cases out of 68 (2.9%)	29 cases out of 122 (23.7%)	3 cases out of 35 (8.5%)

The results in table 3 show that the incidence of false negative of the cytochemical method carried out with cervico-vaginal lavage was higher than that of the vaginal cytology (smears made from sample material taken prior to lavage from the posterior fornix). Better results were attained with endometrial lavage. This technique, however, provided a very high proportion of false positive results: out of 339 patients with no evidence of malignant tumors of the genital organs the results of endometrial lavage were positive in 109 (32,1%) and doubtful in 28 (8,2%).

The causes of false positive results is probably the fact that large hystiocytes of the « foam cells » type are frequently present not only in the stroma of endometrial adenocarcinomata, but also in hyperplastic endometrial tissues and in cervical polyps.

Analysis of false positive results in smears from endometrial lavage:

- a) hyperplasia of the endometrium, 35 cases;
- b) normal endometrium, 23 cases;
- c) atrophy of the endometrium, 18 cases;
- d) endometrial polyps, 9 cases;
- e) fibromyoma of the uterus, 5 cases;
- f) epidermoid metaplasia of the endometrium, 2 cases;
- g) non-specific endometrits, 1 case;
- h) incomplete abortion, 1 case;
- i) hydatiform male, 1 case;
- l) adenoma of the endometrium, 1 case.

In table 4 the results obtained by cytochemical method (cervico-vaginal lavage) are compared with those obtained by vaginal cytology in 48 cases of *malignant tumor of the ovary*.

Table 4. *Diagnostic accuracy of the triphenyltetrazolium chloride method and of cytology in 48 cases of carcinoma of the ovary.*

Results	TTC method		Cytology	
	No of cases	per 100	No of cases	per 100
Positive	9*	18.7	3*	6.0
Doubtful	5	10.4	7	14.5
Negative	34	70.8	38	79.1

\* There were metastases in the vagina in two of these cases.

Ascites was present in 20 out of the 40 cases of malignant tumor of the ovary and the cytochemical test was also performed on the ascitic fluid. Equal parts of the fluid and of the reagent were mixed in a test tube which was then placed in a thermostatic bath at a temperature of 37-38 °C for at least two hours before the sediment was examined. The cytochemical pattern of positivity in the ascitic fluid was similar to that previously described in the cervico-vaginal and endometrial wash-outs. A comparison of the diagnostic value of the cytochemical method and the cytological method in determining the nature of the ascitic fluid from 20 patients with a histologically confirmed malignant neoplasm of the ovary is shown in table 5; for control, the two diagnostic methods were

applied in 32 cases of peritoneal and pleural effusions due to conditions other than malignant tumors (pleuritis, cirrhosis of the liver, cardiac failure, pulmonary embolism).

The table indicates that the cytochemical method tends to give a rather high number of false positive results. As regards false negative results, there was only one case in the 20 examined (5%) in which both diagnostic methods were negative.

The results of the Triphenyltetrazolium chloride (TTC) test compared with the two other methods in 23 cases of *gynaecological cancers at other sites* were as follows:

Table 5. *Comparison of results with the triphenyltetrazolium chloride (TTC) method and cytology in the diagnosis of the nature of effusions (in 52 cases).*

Results	Neoplastic effusion (20 cases)		Non neoplastic effusions (32 cases)	
	TTC method	Cytology	TTC method	Cytology
	No of cases	No of cases	No of cases	No of cases
Positive	18	15	6	1
Doubtful	0	1	9	5
Negative	2	4	17	26

a) both the cytochemical test (on specimens of cervico-vaginal lavage) and vaginal cytology were negative in two cases of tubal carcinoma;

b) the cytochemical method was positive in all eight cases of vaginal carcinoma, while the cytological test was positive in six and doubtful in two and colposcopy was positive in six and negative in two;

c) out of nine cases of carcinoma of the vulva the cytochemical method was positive in two, doubtful in one and negative in six, while cytology and examination with the colposcope were each positive in two, doubtful in four and negative in three;

d) out of the four cases of gestational chorioepithelioma of the uterus (two with metastases in the vagina) the cytochemical method was positive in two and negative in two, while cytology was positive in one, doubtful in one and negative in two.

The cytochemical test was the only diagnostic method used in 1610 out-patients. The object in this group was to evaluate the test as a method of selecting cases for further, more detailed, examination. In all these 1610 cases the test was performed on material obtained by cervico-vaginal lavage. At the time of examination, 750 women were pregnant and 96 were in menopause. As a result four cases of carcinoma of the cervix, three invasive and one in situ, and two cases of endometrial adenocarcinoma were detected. The yield of false positive was 4,3% (69 cases). It was of course not possible to assess the incidence of false negative results.

## COMMENTARY AND CONCLUSIONS

In our experience the advantage of the technique proposed by Ku are as follows:

a) it is extremely simple to perform and to interpret at a low cost; it is possible to train personnel, not skilled in the field of cytology, to read the specimens correctly within a week;

b) it is reasonably accurate in the diagnosis of invasive carcinoma of the cervix, specially if one taken into account the fact that false positive results are almost always associated with some pathological changes which require further investigations;

c) in view of what was stated in b) and of the limited valute of cytology in carcinoma of the endometrium, the test may be helpful in this field;

d) it may be combined with a second diagnostic method in a « two method » screening scheme, because false negative results obtained by the TTC method usually do not coincide with false negative results obtained by other methods.

The drawbacks of the cytochemical test are as follows:

a) it is of little value in the early diagnosis of cervical cancer (51% of false negative results in carcinoma in situ);

b) the specimens cannot be preserved and it is not possible to defer their reading;

c) the test is not specific;

d) the interpretation of smears is rendered difficult by bleeding.

#### SUMMARY

The diagnostic accuracy of the triphenyltetrazolium chloride (TTC) cytochemical method was evaluated by comparison with cytology and colposcopy in 8.491 patients (of whom 548 had gynecological cancer). The method is based on the demonstration of lipids and oxidative enzymes in the cytoplasm of exfoliated cells. False negative results were obtained by the method in 5% of cases of invasive carcinoma and in 51% of cases of carcinoma in situ of the cervix uteri. Using material obtained by endometrial lavage, the incidence of false negative results in carcinoma of the uterine body was low (2,9%), but that of false positive results was very high (32,1%). The test was also evaluated as an exclusive method of screening in 1.610 outpatients; six cases (0,3%) of malignant neoplasms of the genital organs were detected.

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## **Long-term results in the chemotherapy of primary malignant tumours of the ovary**

by

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In this report we discuss the long-term results obtained in the chemotherapy of primary malignant tumours of the ovary.

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