

AN EVALUATION OF CYTOLOGICAL STUDIES AT THE CANCER INSTITUTE WITH REFERENCE TO THE T.P.T. TECHNIQUE*

by

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Introduction

Though the diagnosis of malignancy by the smear technic and crush preparations had been known for some years, it was Papanicolaou's pioneering studies which really established it as a valuable, reliable and standardised procedure in the diagnosis of malignancy. Papanicolaou's work stimulated great interest in exfoliative cytology and numerous other workers have added and amended, so that a large volume of literature has grown on this subject. At the International Cancer Congress at London in 1958 a Japanese Scientist, Dr. Hideo Yagi, presented a new technic and principle of cytological diagnosis. He termed it the "T.P.T." method.

T.P.T. Technic

T.P.T. (2, 3, 5, triphenyltetrazolium chloride) is a colorless crystalline substance soluble in water. A 0.5% solution of this in physiological saline was employed.

A drop of the fluid under examination is placed on a clean coverslip, a drop of the T.P.T., solution is added and mixed well. A slide is next

inverted over the suspension to get a thin uniform film. The edges of the coverslip are sealed off with vaseline. The slide is kept at room temperature (29°-32°C. at Madras) for about 20 minutes to half an hour and then examined under both the low and the high power of a microscope. Cells containing well-stained red granules are taken as "positive", colourless cells as negative. Occasionally, cells appear which contain a very few granules — these may be classed as doubtful.

The entire basis of the T.P.T. method seems to depend on its enzymatic reduction to a water-insoluble red coloured compound, formazan, in the cell. The enzymes appear to exist in high concentration in the cancer cells which have a higher rate of anaerobic glycolysis than non-cancer cells.

Reason for This Study

The T.P.T. method is not widely known. It appeared simple in principle and execution, it consumed little time and did not seem to need any special training as was necessary in the Papanicolaou method. We had, of course, to check its reliability.

In India, the number of gynaecological clinics are few compared to its enormous population. The num-

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ber of gynaecological patients seen at the out-patients each day is phenomenal. The cost of Papanicolaou method is high compared to the T.P.T. method. The T.P.T. method seemed more suited for this mass screening and also appeared cheaper.

Material

The material reported on in this paper consists of 162 patients seen at the Institute over a one-year period for either vaginal bleeding or leucorrhoea.

The T.P.T. method was also tried out in bronchial aspirations, serous effusions, urinary sediments, discharges from the nipple and from sinuses. These, however, are not analysed in this series.

Method of Study

Our study has differed from others in that we assumed clinical diagnosis to be the firmest criterion against which the other methods were checked. In those cases where the clinical diagnosis agreed with the biopsy and cytological diagnosis no problems arose. But where the clinical diagnosis differed from the biopsy and cytological diagnosis we preferred to rely on the clinical diagnosis; and the further course of the patient's condition proved us correct except in one case. This case will be referred to again during the discussion.

By biopsy we mean one or more bits of tissue removed from the uterine cervix from a single or more sites with a specially designed punch and subjected to paraffin sectioning and microscopy. This is not to be confused with the serial sectioning of the cervix that was carried out

after the modified Wertheim's hysterectomy in the clinically negative but cytologically positive cases.

Fluid from the posterior fornix was aspirated with a slightly curved glass pipette after the introduction of a speculum, the first thing in the morning, for the T.P.T. study. The technique of cervical rub was used for the Papanicolaou smear.

The method of biopsy, Papanicolaou's smear study and the T.P.T. study were carried out by different individuals *on each case* and independent records were kept. After the completion of the series they were separately checked against the clinical records. This was done to prevent any bias in reporting.

Analysis

TABLE I

	Absolute No.	Percentage
Clinically proven cancer of the uterine cervix	128	..
No. of cases in which biopsy was done	124	100
Biopsy positive for cancer	115	92.7
No. of cases in which Papanicolaou was done	125	100
Papanicolaou positive for cancer	114	91.2
No. of cases in which T.P.T. was done	128	100
T.P.T. positive for cancer	115	89.8

TABLE II

	Absolute No.	Percentage
Clinically proven cancer of the uterine cervix	128	..
Biopsy false negative	7/124	5.6
Papanicolaou false negatives	7/125	5.6
T.P.T. false negatives	13/128	10.1

TABLE III

	Absolute No.	Percentage
Clinically proven negative for cancer	34	..
Biopsy false positive		nil
Papanicolaou false positive	3/34	8.8
T.P.T. false positive	3/34	8.8

Commentary

It is our opinion that the most reliable diagnosis is always clinical supplemented by biopsy. This is obvious from the foregoing tables.

Of the 162 cases under report there was not a single clinical misdiagnosis. In over 1200 cases of cervical carcinoma we can recall only one in which a clinically negative case was discovered to contain a carcinoma tucked away in the endocervix (incidentally cytology was positive for carcinoma in this case, though repeated biopsy was negative.) The corresponding accuracy in positive carcinoma diagnosis for biopsy was 92.7%, for Papanicolaou 91.2%, for T.P.T. 89.8%. The false negative was none for clinical, 5.6% for biopsy, 5.6% for Papanicolaou and 10.1% for T.P.T. (Tables I & II).

Apart from the all-important clinical diagnosis, there appears very little to choose statistically between the biopsy and the two cytological methods from the point of view of diagnosing existing malignancy. The figures are certainly slightly in favour of the biopsy.

Of the 162 cases, 34 were proved free from cancer of any generative organ. While the biopsy agreement was 100%, Papanicolaou agreement was only 91.2% and T.P.T. agreement

was 91.2%. Again, while the biopsy false positives were nil, the Papanicolaou false positives were 8.8%, and T.P.T. false positives were 8.8% (Table III). It would appear from this table that in the non-cancer cases with some inflammatory gynaecological pathology errors in cytology creep in quite easily which is rather difficult for the average pathologist to detect. The biopsy seems to be the most reliable.

The overall picture, therefore, is weighted heavily in favour of the age-old clinical diagnosis and the biopsy.

It is, however, difficult to take up rigid positions. As we have already said there was one case where the diagnosis would have been completely missed in the pre-Papanicolaou days. The detection of even this single additional case has improved our accuracy of diagnosis and made it possible to save one more life.

In two cases of a sero-sanguinous discharge from the nipple no tumour could be palpated in the breast. A needle biopsy would certainly have been a failure. A simple mastectomy and serial sectioning of the breast was unthinkable. But the smear in each case revealed an abundance of malignant epithelial cells, some of them in mitosis.

In two of our cases of a radical neck dissection for metastatic lymph nodes a small pocket of fluid with a peripheral zone of induration developed, one in the submandibular region and the other in the retro-mandibular region, eight weeks after the operation. We could not be certain whether the lesion was inflammatory or the result of a malignant

infiltration. Biopsy would have been very unwelcome, but aspirated fluid and smear demonstrated malignant cells.

Our growing experience seems, therefore, to suggest that each method has its advantages and its fallacies. In cases where a radiogram rouses the suspicion of a bronchogenic carcinoma but bronchoscopy reveals no tumour which can be biopsied, in the types of occult duct carcinoma of the breast which we have quoted, in the small hidden primary in the cervix or endometrium, these are cases in which cytology plays a very helpful role. As we have also pointed out earlier it is also very easy to identify a cancer cell where none exists. A positive cytology report for cancer in the presence of a negative histological and clinical diagnosis should be accepted with caution and checked very carefully. It would be unwise to neglect it altogether but at the same time hasty if one assumed it to be conclusive.

The cytological method had also been found to be very useful in the checking of our follow-up cases. We hesitate a great deal to inflict the trauma of a biopsy on recently irradiated healed tissues for a variety of reasons. The smear comes in very handy.

Statistically, there is nothing much to choose between the Papanicolaou smear technic and the T.P.T. film technic. The percentage accuracy figures in the positive diagnosis of malignancy are close (91.2% and 89.8%), as also in the false positives and negatives. In the breast, neck and uterine cases which we have quoted individually both Papanicolaou and T.P.T. read identically,

both in their correctness and in their errors.

The T.P.T. has certainly the advantages of being simpler, quicker, cheaper and more unequivocal. The percentage of 'doubtful' cases was only 3% in T.P.T. but 7.5% in Papanicolaou (in biopsy it was only 1.8%). It can be carried out with very little paraphernalia in a large gynaecological outpatient in a corner by a technician trained to use a microscope but without any extra special cytological training. Papanicolaou, of course, provides information other than malignancy regarding epithelial cells and certain physiological processes. But in the cytological identification of malignancy we have certainly found the T.P.T. film technic more rapid, easier and more dependable. However, it should not be forgotten that the clinical diagnosis and the biopsy are paramount and still unsurpassed.

Summary

An attempt has been made to evaluate relative values of the biopsy, the Papanicolaou and the T.P.T. methods in the diagnosis of cervical carcinoma. The study seems to indicate that though clinical diagnosis and the biopsy have the best all-round fidelity in positive and negative diagnosis, the cytological methods are also quite dependable. The cytological methods have their greatest value where a biopsy seems impossible because the primary is unidentifiable, as in some cases of carcinoma of the lung or the breast or in a very small endo-cervical carcinoma or a carcinoma in situ and in the follow-up cases. On the other hand,

the cytological methods are also susceptible to fallacies.

Between the Papanicolaou and the T.P.T. there is not much to choose, but the T.P.T. is cheaper, easier, more rapid and more unequivocal. We feel that it is better suited to a country like ours.

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Reference

Yagi H.: Acta un- int. Cancer; 15 (2), 349-353, 1959.