Recent Developments in the Cytologic Diagnosis of Vaginal, Endometrial and Ovarian Cancer

BERNARD GONDOS, M.D.

Department of Pathology, University of California, San Francisco, CA 94143

ABSTRACT

Cytology has proved to be an effective and simple technique for the diagnosis of cancer of the uterine cervix. The usefulness of cytologic methods in detecting other types of tumors of the female genital tract has been relatively limited. In recent years, new developments have focused attention on the use of cytology in the following areas: (1) detection of adenosis and adenocarcinoma of the vagina in daughters of women who received synthetic estrogens during pregnancy; (2) detection of early stages of adenocarcinoma of the endometrium by direct sampling methods and (3) prognostic evaluation of cancer of the ovary by examination of peritoneal washings. The present report reviews the cytologic methods involved in these procedures with consideration of both technical and interpretive aspects.

Introduction

Cytologic diagnosis of cancer of the uterine cervix has achieved great success over the past 25 years. Detection of cervical neoplasia in the stages of dysplasia, carcinoma in situ and early invasive carcinoma has enabled treatment and cure of a potentially fatal disease in numerous cases. Evidence of this is the marked decline in mortality from cancer of the cervix in the past two decades.

Unfortunately, the same cannot be said for cancer of the endometrium and ovary, the two other common sites of malignancy in the female genital tract. Endometrial cancer has shown a steady increase in incidence in recent years. Although the reasons for this are not known, it is evident that traditional cytologic methods have been ineffective in preventing the rise. Similarly, cancer of the ovary remains a difficult diagnostic problem. The inaccessible location of the ovaries makes cytologic sampling particularly difficult and new approaches in this area are badly needed.

Cytologic diagnosis of vaginal cancer presents a different type of problem. Clarification is required on the role of cytology in regard to the newly recognized conditions of adenosis and adenocarcinoma of the vagina occurring in young women whose mothers received synthetic estrogens during pregnancy. Because of the extremely high number of possibly affected individuals and the serious implications in-
volved, early recognition of these lesions is of special concern. The possible contribution of cytology as a means of early detection is an important consideration.

Cancer of the Vagina

Primary carcinoma of the vagina is an uncommon tumor and one which, until recently, had been found to occur predominantly in the sixth and seventh decades in the form of squamous cell carcinoma. In 1970, Herbst and Scully\textsuperscript{16} described seven cases of adenocarcinoma of the vagina occurring in adolescents. Detailed investigation in these cases suggested an association with maternal administration of stilbestrol and other synthetic estrogens. This association was further documented as additional cases were collected.\textsuperscript{12,17,32} It thus became clear that detection of adenocarcinoma of the vagina in daughters of women who received synthetic estrogens during pregnancy represents an important diagnostic problem.

Early studies indicated that routine cytologic evaluation was not reliable in the diagnosis of vaginal adenocarcinoma. Of 30 cases in which specimens were obtained for cytology, 11 were positive, nine negative and 10 doubtful.\textsuperscript{15} It was subsequently reported by Vooijs et al\textsuperscript{33} that cytology was useful in the detection of vaginal adenocarcinoma and its precursor, adenosis, when direct vaginal scrapings were taken. These studies indicate that the manner in which specimens are obtained is critical. Routine sampling of cervix and vaginal pool will fail to detect many cases of adenosis and adenocarcinoma, while direct sampling of vaginal lesions can be of diagnostic value.

Recognition of vaginal adenosis is especially important because of its probable role in the pathogenesis of adenocarcinoma.\textsuperscript{15} Although a direct relationship remains to be established, the frequent coexistence of adenosis in cases of adenocarcinoma supports such a relationship. Adenosis is characterized by the presence of regular single-layered glandular structures located beneath the stratified squamous mucosa. The process may extend to the mucosal surface. The glands are generally lined by tall columnar endocervical-type epithelium with basally situated nuclei and abundant cytoplasm (figure 1). These cells are usually PAS-positive. The cytologic diagnosis of adenosis is indicated by the presence of cells resembling endocervical epithelium or by the presence of endometrial-type cells. Vaginal adenosis can be detected by direct scraping of lesions which appear as reddened granular areas, most often found in the upper anterior vaginal wall. In the absence of a visible lesion, occult adenosis may be detected by circumferential scraping of the vaginal mucosa. In doing this, it is important to minimize the possibility of contamination from the endocervix by removing any secretion overlying the vaginal mucosa before performing the scraping. The presence of endocervical-type cells can then be taken as evidence of vaginal adenosis.

Adenocarcinoma of the vagina appears as a grossly evident tumor usually, but not always, located in the upper anterior vagina. The size is variable, ranging from one to ten cm in diameter. Histologic appearance has been reviewed in detail elsewhere.\textsuperscript{16} The principal features are a well differentiated glandular pattern with clear cells lining the gland spaces (figure 2). The pattern may be solid in some cases but is most frequently cribriform or "hobnail" type. At higher magnifications, the cells can be seen to have slightly irregular nuclei and large nucleoli (figure 3A). Cytologic diagnosis depends on direct sampling of the tumor. The cells exhibit characteristic features of adenocarcinoma, including aggregation in groups, increased nucleocytoplasmic ratio and marked nucleolar enlargement (figure 3B). Slight to
Figure 1. Vaginal adenosis with characteristic endocervical-type epithelium. Hematoxylin and eosin, x400.

Figure 2. Vaginal adenocarcinoma, hob-nail pattern. Hematoxylin and eosin, x80.

Figure 3. Vaginal adenocarcinoma in a 15-year-old girl whose mother had received stilbestrol during pregnancy. Both histologic and cytologic preparations show cell groups with prominent nucleoli, slight nuclear variability, relatively homogeneous chromatin pattern and clear to finely granular cytoplasm. A. Tissue section; Hematoxylin and eosin, x400. B. Cytologic smear; Papanicolaou stain, x500.
Cytologic Diagnosis of Adenosis and Adenocarcinoma of the Vagina

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Specimen</th>
<th>Stain</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosis</td>
<td>Obtain scrapings from lesion; if no lesion present, scrape vaginal mucosa circumferentially, avoiding cervical contamination.</td>
<td>Routine Pap stain (PAS may be helpful)</td>
<td>Strips or sheets of benign columnar cells resembling endocervical or endometrial cells; basal nuclei, vacuolated cytoplasm, occass. Ciliated, may be PAS positive.</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>Obtain scrapings from lesion.</td>
<td>Routine Pap stain</td>
<td>Groups of neoplastic cells with pleomorphic round to oval nuclei, multiple irregular macronucleoli, finely granular faintly eosinophilic cytoplasm.</td>
</tr>
</tbody>
</table>

Since the diagnosis of vaginal adenocarcinoma by cytologic examination has thus far been made only in clinically evident lesions, it remains to be established whether or not a preclinical stage of adenocarcinoma can be detected. In the meantime, it is recommended that vaginal scrapings be employed routinely in the examination of adolescents whose mothers received stilbestrol or other synthetic estrogens during pregnancy. Cytology can also be used in following patients with a diagnosis of vaginal adenosis. Until the extent of the relationship between adenosis and adenocarcinoma is more fully known, careful cytologic follow-up will remain a principal means of managing patients with adenosis.

Cancer of the Endometrium

Endometrial cancer has shown a sharp rise in recent years. Although improved detection may account in part for the increasing incidence, failure to reduce the mortality indicates that early detection is not being achieved. Menopausal and post-menopausal women are the prime suspects, but cases in women under 40 are being increasingly reported. In addition, there is a general rise in the most aggressive form, adenosquamous carcinoma.

The traditional method for cytologic diagnosis of endometrial lesions is sampling of the vaginal pool. This method relies on exfoliation of cells from the endometrial lining into the region of the vaginal fornix. Because this is an indirect form of sampling, the false negative rate is high, especially so for early lesions. The endometrial cells encountered in vaginal pool material are often in a poor state of preservation; differentiation of degenerated endometrial cells, histiocytes, distorted endocervical cells and cells from endometrial hyperplasia and adenocarcinoma may be difficult. As a result, routine screening has contributed only rarely to the diagnosis of endometrial carcinoma.

A variety of methods for direct sampling of the endometrium have been utilized in an attempt to overcome these problems. The techniques involve aspiration, brushing or lavage. Comparison of the advantages and disadvantages of the different methods is shown in table II.
TABLE II

Comparison of Endometrial Cytologic Techniques

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal pool</td>
<td>Simple; inexpensive.</td>
<td>Indirect sampling depending on cellular exfoliation; high false negative rate; difficult to interpret.</td>
</tr>
<tr>
<td>Aspiration</td>
<td>Simplest of the direct methods; good cellular yield; good cell detail.</td>
<td>Specimen may be bloody; possibility of perforation.</td>
</tr>
<tr>
<td>Brushing</td>
<td>Good cellular yield.</td>
<td>Expensive; involved procedure; specimen may be bloody; possibility of infection.</td>
</tr>
<tr>
<td>Lavage</td>
<td>Good cellular yield.</td>
<td>High false positive rate in early reports; involved procedure; expensive; possible spread of malignant cells.</td>
</tr>
</tbody>
</table>

the aspirated material is centrifuged to form a cell button from which smears and histologic sections can be prepared. The accuracy of aspiration has ranged from 70 to 90 percent in different studies. Major disadvantages include the risk of perforation, the frequent admixture of excessive blood and the requirement that the procedure be performed by a specialist.

Endometrial brushing, employing a variety of brushes for obtaining direct smears, has an accuracy of 57 to 92 percent in cases of histologically confirmed adenocarcinoma. The technique is too involved and expensive for routine use and has the additional drawback of possible introduction of infection.

Lavage techniques involve irrigation of the uterine cavity with saline and collection of the fluid in centrifuge tubes. After the material is centrifuged, smears and cell blocks can be prepared from the sediment. Various types of cannulas have been used to collect specimens. All of the techniques have the potential risk of forcing malignant cells into the peritoneal cavity via the fallopian tubes. There may also be considerable difficulty in interpretation resulting in a significant false positive rate. The accuracy of different lavage techniques has been reported to range from 80 to 90 percent.

Recently, a new endometrial lavage technique employing the Gravlee jet washer has been recommended as an effective screening technique with a high degree of accuracy. This method utilizes a disposable double lumen cannula, through which 30 to 40 ml of saline are washed to collect material in a plastic container. The application of suction by a syringe in one tube creates a vacuum, exerting negative pressure and thereby avoiding spread of malignant cells. With this technique, smears, millipore filters and cell blocks can be prepared, the latter frequently containing tissue fragments. The procedure is involved and expensive, but has received considerable use. Reports on results are conflicting. Some have been enthusiastic about the lack of technical problems and high detection rate, while others have found a considerable number of specimens to be unsatisfactory, particularly in postmenopausal women. There is general agreement that the technique compares favorably with endometrial biopsy in the presence of abundant neoplastic tissue, but its use as a screening procedure in asymptomatic women has not been universally supported.

Of all the methods for direct endometrial sampling, endocervical aspiration appears to be the simplest and the one most widely employed. This technique can be utilized as part of the gynecologic examination in conjunction with routine screening for cervical disease. Ng et al have recently described the use of endocervical aspirates to evaluate cytologic

* Upjohn Company, Kalamazoo, MI 49001.
criteria for diagnosing precursors of endometrial cancer, such as adenomatous hyperplasia, atypical hyperplasia and adenocarcinoma in situ. They were able to establish satisfactory criteria for distinguishing these conditions from endometrial carcinoma, although agreeing with others that even with the aspiration technique interpretation is difficult and requires considerable experience. The technique clearly provides a more constant and greater concentration of endometrial cells than traditional indirect sampling techniques and, with increasing experience, may prove to be effective in detecting precursors of endometrial cancer.

Cancer of the Ovary

The overall survival rate for ovarian cancer has not changed appreciably in over a quarter of a century, largely owing to late diagnosis. Attempts to utilize cytologic detection have been ineffective. Again, the problem is one of providing a direct method of sampling that is simple and reliable.

Culdocentesis, or cul-de-sac aspiration, has been used as a method for detecting cells from ovarian tumors over the past decade. In 1964, Graham et al. reported on a series of 516 cul-de-sac aspirations obtained from asymptomatic volunteers. There were eight positive cases, of which seven were explored: one had metastatic breast cancer, four had papillary lesions in the ovary of "borderline malignancy," one had a "probable borderline lesion" and one had no demonstrable lesion. In reviewing these findings, Koss suggested that the method of culdocentesis required further evaluation and should be given a wide field trial. Subsequently, over 1,400 asymptomatic women were screened in two separate studies with only one positive diagnosis. A serious problem of the method was in obtaining sufficient cellular material. Satisfactory specimens were obtained in only 70 percent of aspirations, and in many others the cellular yield was minimal.

Furthermore, the question has been raised as to whether or not it is too late for effective therapy when malignant cells are already present in the cul-de-sac washings. Graham has expressed the opinion that the presence of malignant cells in the aspirate does not necessarily mean advanced disease, since the cells might be exfoliated without implantation on the peritoneal surface. On the other hand, many of the common ovarian tumors of the surface epithelium remain confined within the ovarian parenchyma during their early growth and exfoliation into the peritoneal cavity would be a late event in these cases.

Peritoneal washings obtained at laparotomy have also been used in the diagnosis of ovarian cancer. Creasman and Rutledge reported on a ten year experience with more than 1,200 specimens. Most were obtained in cases of known ovarian cancer for prognostic evaluation. Also included was a group of 112 patients who were operated on owing to benign gynecologic disease. One hundred to 150 cc of saline were instilled into the peritoneal cavity and allowed to collect in the pelvic region. The fluid was then removed and treated in the usual manner for cytologic study, smears and cell blocks being prepared from the spun-down sediment. Abnormal cells were reported in five of the asymptomatic patients, none of whom was found to have malignant disease. However, three had ovarian cystadenomas and two had benign conditions involving the uterus. These results represent a significant false positive rate and point out the hazards of interpretation in peritoneal washings. In the patients with known ovarian cancer, those with abnormal cytologic findings had survival rates considerably lower than those with normal findings. Cytology was therefore considered useful in prognostic
assessment and evaluation of further treatment.

For the present, it appears that cytology has little to offer in the initial diagnosis of ovarian cancer, but it can be utilized for the purpose of prognostic evaluation through the use of peritoneal washings.

References


---

**Manual of Procedures for the Applied Seminar on the Laboratory Diagnosis of Skeletal, Muscular and Nervous Disorders**

The following procedures are included in this listing:

- Direct Measurement of Serum Calcium
- Direct Measurement of Serum Inorganic Phosphate
- Practical Aspects of Radioimmunoassay of Parathyroid Hormone
- Radioimmunoassay for Human Parathyroid Hormone
- Measurement of Alkaline Phosphatase in Serum
- Determination of Serum Alkaline Phosphatase Isoenzymes
- Measurement of CPK Isoenzymes in Serum
- Hypoxanthine-guanine Phosphoribosyltransferase (HGPRT) Assay
- Detection of Tay-Sachs Heterozygotes by Determination of β-N-Acetyl-D-Hexosaminidase A
- Measurement of Blood Lactate
- A Kinetic Colorimetric Method for the Measurement of Uric Acid in Serum
- A Kinetic Uricase Method for Measurement of Uric Acid
- Measurement of Salicylates in Serum
- Measurement of Serum Gold in the Regulation of Chrysotherapy of Rheumatoid Arthritis
- Measurement of Folic Acid and Vitamin B₁₂ in Serum
- Measurement of Homovanillic Acid in Cerebrospinal Fluid
- Latex and Sheep Cell Agglutination of Rheumatoid Factor
- Methods in the Immunofluorescent Diagnosis of Connective Tissue Diseases
- Streptococcal Antibody Tests (Antistreptolysin O, Antihyaluronidase, AntiDNase B, Antistreptokinase, AntiNADase)
- Electroimmuno Assay Procedures for the Measurement of Orosomucoid, Alpha-₁-Antitrypsin and Ceruloplasmin
- Evaluation of Acute Phase Reactants
- The Fluorescent Antibody Test for Toxoplasmosis
- Glutamine and Ammonia Measurements in Cerebrospinal Fluid
- The Laboratory Diagnosis of Galactosemia
- Measurement of Acid Mucopolysaccharides
- Measurement of Serum Dilantin and Other Antiepileptic Drugs
- Measurement of Serum Lithium in the Regulation of Lithium Therapy
- Protein Measurements in Cerebrospinal Fluid
- Polycrystalline Gel Electrophoresis of Spinal Fluid Proteins
- Measurement of Lead in Biological Fluid
- Sensitive Determination of Urinary Coproporphyrin and Uroporphyrin by Chromatography and Fluorometry
- Thin Layer Chromatography for Urinary Porphyrins
- Serologic Tests for Viral Myocarditis
- Needle Biopsy of Bone Marrow

Published by the Institute for Clinical Science, Inc. for the Association of Clinical Scientists

**Special Price $13.00**

Order directly from: Institute for Clinical Science, Inc., F. William Sunderman, M.D., Director, 1833 Delancey Place, Philadelphia, Pennsylvania 19103