Newer Techniques in Clinical Cytology: Aspiration Biopsy and Brushing Cytology*

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Since its introduction some twenty years ago the Papanicolaou technique for cervical-vaginal cytology has had a tremendous impact on the detection of precancerous lesions and in situ cancer in the female genital tract. It has forced the clinician and pathologist to study the “early lesions” of cancer, particularly in the female genital tract and has brought about a better understanding of their biology. Cytology has over the years been extended to other areas, particularly the respiratory tract. Two recent areas of emphasis are the thin-needle aspiration biopsy and direct brushing of mucosal surfaces through the various fiber optic scopes now available, particularly for the respiratory and gastrointestinal tract.

Thin-Needle Aspiration Biopsy. This is an old technique having been employed at Memorial Center for Cancer and Allied Diseases for over thirty years. It has never become popular in the United States, but in the last ten or fifteen years has become widely used in Europe, particularly in the Scandinavian countries. At the Karolinska Institute, approximately 12,000 such aspirations are done per year, mainly for mass lesions of the breast, prostate, lung, thyroid and lymph nodes. The technique renders a rapid diagnosis, particularly on benign and malignant tumors, and is usually done on an outpatient basis. In the setting described, a saving of time and expense to the patient is obvious.

The technique of thin-needle aspiration is done with a narrow gauge 22 or 23 needle of various lengths, percutaneously, without anesthesia. Because of the nature of the needle it is nontraumatic and less painful than a venipuncture. Important is the use of a syringe such as the Franzen type, which may be operated with one hand (11). The other hand is used to fix the tumor mass and thereby control the needle point. The type of syringe holder that will employ disposable 20 ml plastic syringes is illustrated in figure 1.1

The needle attached to the syringe is introduced into the mass to be aspirated without the application of vacuum. The vacuum is applied and the needle is moved back and forth in the mass several times. The operator should watch the barrel of the syringe and avoid getting material into the barrel. It is not necessary to obtain a core of tissue, but if tissue fragments are obtained that is an added bonus. The idea is to obtain cells and to keep the material within the needle.

If a cyst is aspirated this may be evacuated by filling the syringe. If the fluid is clear it is unrewarding to process it either as a smear from the cell button of the centrifuge specimen or by one of the filter techniques, either nucleopore or millipore. If the fluid is cloudy or hemorrhagic it is advisable to process it as described, either by filter techniques or smears from the cell button. Cell blocks are not useful but any fragments of tissue may be processed as a biopsy.

Once the needle has been moved back and forth in the mass several times the pressure in the syringe is allowed to equalize before the needle is removed from the mass. This is extremely important.

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1 The Cameco Syringe Pistol® purchased from Precision Dynamics Corporation, 3031 Thornton Ave., Burbank, California 91504.
It prevents a chance to deposit the cells along the needle tract. The syringe is then withdrawn and the needle is held over plain glass slides while the syringe is removed. Air is then introduced into the syringe, the needle reattached and a small drop or two of material is expressed onto the slides. There is usually enough material to make two or three slides. Smears are prepared much in the manner of making a blood smear, but the entire slide need not be covered and the material may be confined in a smaller area. Smears to be stained by the Papanicolaou technique should be immediately fixed while still wet in methyl alcohol. Smears for May-Grunwald Giemsa stain or Wrights-Giemsa or hematoxylin and eosin are allowed to air dry. In addition, on air dried smears we have used a metachrome B stain with success. This is the same stain that we employ on frozen section material. Smears prepared in this way may be made permanent by simply washing the metachrome B stained slide with water, allowing this to evaporate and then coverslapping with Permount®.

European investigators have favored the hematology stains while pathologists in the United States have favored the hematoxylin and eosin or Papanicolaou stain. I have found the metachrome B stain combined with the Papanicolaou stain to be extremely useful.

In the aspiration biopsy technique using the thin-needle, the clinical evaluation is extremely important. Since the material is small in amount and of a cellular type rather than a tissue fragment, clinical evaluation of the mass and its orientation becomes of utmost importance. The greatest success with this technique has been when the pathologist who is reading the aspiration has also taken the material. He has palpated the tumor mass and has a first hand knowledge of the clinical situation. If clinicians wish to adopt this technique and send their material to be read by a pathologist, then they must supply extremely detailed clinical information to avoid errors, especially of the false positive type. The technique has been used particularly to aspirate lymph nodes with metastatic tumors, for breast tumors, thyroid lesions, salivary gland tumors, lung tumors through the transthoracic route, prostate and kidney tumors and soft part sarcomas. There has been limited use in bone tumors. Prostate aspirations are done transrectally with a metal guide which fits over the examining finger and is so positioned that the tip of the needle will pass into the mass palpated by the tip of the examining finger.

Results. Results from several selected sites in a large series are summarized in Table 1. One of the most useful areas has been cervical lymph nodes, particularly for metastatic tumors. It can be seen that in a large series of cases there were no false positive reports and only a small percentage of false negative reports. Primary sites of these metastatic tumors were equally divided between those above the clavicle and those below (11). In 35% of these cases the aspiration was the first indication of cancer (11). Zajicek, et al. (27), obtained similar results in 1,200 consecutive aspirations of cervical nodes.

Two difficult areas are congenital cysts of the neck and carotid body tumors. Engzell and Zajicek (8) reported the aspiration of 100 consecutive congenital cysts of the neck and compared these with 100 aspirations of cases of metastatic squamous cell carcinoma. They made a definite diagnosis from the aspiration in 83% of the cysts and in 95% of the carcinomas. The diagnostic accuracy varied with the
TABLE 1
THIN-NEEDLE ASPIRATION BIOPSY RESULTS IN SELECTED SITES

<table>
<thead>
<tr>
<th></th>
<th>No. of Cases</th>
<th>Correct Diagnoses</th>
<th>False Positive</th>
<th>False Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head and Neck</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical Lymph Nodes (11)</td>
<td>257</td>
<td>93.7%</td>
<td>0</td>
<td>6.3%</td>
</tr>
<tr>
<td>Salivary Gland Tumors (4, 5, 6, 7, 26)</td>
<td>368</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign Mixed Tumor</td>
<td>(215)</td>
<td>93.0%</td>
<td>4.0%</td>
<td>3.0%</td>
</tr>
<tr>
<td>Malignant Mixed Tumor</td>
<td>(7)</td>
<td>100.0%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adenoid Cystic Carcinoma</td>
<td>(45)</td>
<td>66.0%</td>
<td>0</td>
<td>33.0%</td>
</tr>
<tr>
<td>Acinic Cell Carcinoma</td>
<td>(34)</td>
<td>65.0%</td>
<td>0</td>
<td>35.0%</td>
</tr>
<tr>
<td>Warthin’s Tumor</td>
<td>(45)</td>
<td>80.0%</td>
<td>0</td>
<td>20.0%</td>
</tr>
<tr>
<td>Oncocytoma</td>
<td>(4)</td>
<td>100.0%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mucoepidermoid Carcinoma</td>
<td>(18)</td>
<td>62.0%</td>
<td>0</td>
<td>38.0%</td>
</tr>
<tr>
<td>Thyroid (3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>(177)</td>
<td>94.0%</td>
<td>0</td>
<td>6.0%</td>
</tr>
<tr>
<td>Cystic Nodules</td>
<td>(61)</td>
<td>98.0%</td>
<td>0</td>
<td>2.0%</td>
</tr>
<tr>
<td>Struma</td>
<td>(20)</td>
<td>100.0%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Breast (15)</td>
<td>1429</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign Nontumor</td>
<td>(305)</td>
<td>88.0%</td>
<td>0.2%</td>
<td>12.0%</td>
</tr>
<tr>
<td>Benign Tumor</td>
<td>(251)</td>
<td>89.0%</td>
<td>0</td>
<td>11.0%</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>(873)</td>
<td>89.2%</td>
<td>0</td>
<td>10.8%</td>
</tr>
<tr>
<td>Prostate (12)</td>
<td></td>
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<td></td>
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<tr>
<td>Carcinoma</td>
<td>101</td>
<td>93.6%</td>
<td>2.4%</td>
<td>4.0%</td>
</tr>
<tr>
<td>Lung (2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>(101)</td>
<td>89.0%</td>
<td>0</td>
<td>11.0%</td>
</tr>
<tr>
<td>Benign conditions</td>
<td>(24)</td>
<td>40.0%</td>
<td>5.0%@</td>
<td>55.0%</td>
</tr>
</tbody>
</table>

* 5.3% of cases suspected as carcinoma were all histologically proven fibroadenomas
@ one case

location of the cyst, being only 55% in medial cysts and 92% in lateral cysts. They found benign looking squamous cells in 6% of the smears from metastatic carcinoma.

Engzell, et al. (10), have reported their experience with 13 cases of aspiration of carotid body tumors. In one case, there were no cells and in two, metastasis was suspected from an unknown primary. Three cases were diagnosed as neurofibroma or neurofibrosarcoma and in seven cases a correct diagnosis of carotid body tumor was made. The authors indicate some caution in aspirating this tumor when it is suspected, as one of their cases became hemoplegic following the procedure.

Aspiration of salivary gland tumors has generally been quite successful by this technique as indicated in Table 1 (4, 5, 6, 7). Adenoid cystic carcinoma, acinic cell carcinoma and mucoepidermoid carcinoma carry fairly high false negative rates (26). Follow-up on benign mixed tumors indicates no increase in recurrence rate due to the aspiration technique. In seven cases following resection, the needle tract was serially sectioned and no tumor cells were found (5). Important in the aspiration smears in the diagnosis of adenoid cystic carcinoma are mucinous globules. These were present in 22 of 45 patients (6). While the false negative rate for adenoid cystic carcinoma over the 24-year period of the original study is high, it has improved over the last 11 years, being only one out of 23 aspirations or 4% (6). This is true also for 34 cases of acinic cell carcinoma in which aspirations of the last 11 years have resulted in a false negative rate of two in 16 or 12% (7).

Other salivary gland carcinomas are too infrequent to document the reliability of the technique. The most difficult has been mucoepidermoid tumors of low grade malignancy. In the report by Zajicek and Eneroth (26), 18 cases of mucoepidermoid carcinoma yielded only cystic fluid, cell detritus and lymphocytes. Poorly differentiated mucoepidermoid carcinoma as well as the unclassified adenocarcinomas were easily identified by the aspiration technique.

Clinicians have been reluctant to aspirate thyroid lesions, but this has been done with considerable success at the Karolinska Institute. Results indicate
no false positives in carcinoma, cystic nodules or struma, and a small percentage of false negatives in carcinoma and cystic nodules (3).

The major area of interest in the United States has been needle aspiration of the breast. Franzen and Zajicek (15) have attained a high degree of accuracy with this technique, not only with carcinoma, but with the benign disease. A conservative attitude is required in dealing with breast lesions since radiation or surgical treatment is frequently done on the basis of aspiration diagnosis. False negative aspirations are generally on the basis of tumors less than 1 cm, very fibrotic, poorly cellular carcinomas or failure to aspirate the residual mass after evacuating a cyst. With increased experience in the recent series, the false negative rate has been cut to 5%.

It can also be seen from Table 1 that the aspiration technique is highly accurate in prostate carcinoma. Since the approach is transrectal, there have been a few cases, less than 2%, of gram negative septicemia attributable to the aspiration (12).

The lung has been the most recent area where the thin-needle technique has been used. Lesions of the lung yield grudgingly to diagnostic techniques of exfoliative cytology or conventional bronchoscopy and biopsy. Prior to the thin-needle technique, a large bore-type needle was used with a frequent tendency to hemorrhage, pneumothorax or air embolism. Dahlgren (2) has used the thin-needle technique under fluoroscopic control with only topical skin anesthesia. In 101 malignant tumor cases, there have been no false positives and in the 24 benign conditions, only one false positive. In the total series of 3,000 thin-needle aspirations, there have been no complications (2). Sputum cytology in the same material has yielded only a 40% diagnostic rate. Nasiell (22) correctly diagnosed from 83 lung cancer cases, 72% by the thin-needle technique. About half of the false negative cases had inadequate material. The results were better than conventional sputum cytology. The technique is thought by that author to be particularly good for peripheral and small tumors or for clinically inoperable cases. The author reported no complications. Sanders, et al. (25), also reported a large series of 164 patients with an accuracy of 84% in malignant neoplasms. The authors did not find the technique accurate in nonmalignant localized parenchymal disease and not particularly good in chest wall or diffuse parenchymal disease. They did find it helpful in the mediastinal mass lesions, particularly pericardial, bronchogenic or thymic cysts. They listed the contraindications as: 1) hemorrhagic diathesis, 2) a patient on anticoagulants, 3) severe pulmonary hypertension, 4) pulmonary hydatid cyst, 5) uncontrolled cough, 6) advanced emphysema, 7) patient with suspected arteriovenous malformation.

These authors used the large bore needle (2.1-3.0 ml) and had a 30% complication rate of small pneumothoraces. This needle is twice as large as that recommended in the thin-needle technique from the European workers (25). Most of these pneumothoraces were asymptomatic. Five percent in this series, however, required tube drainage. In the follow-up of this series, there were no verified cases of tumors spread by the needle technique employed.

Janower and Land (20) also used the thicker needle of the Vin-Silverman type. As expected, they had a 10-15% minimal complication rate of hemoptysis and pneumothorax. These larger needles cannot be recommended any longer, therefore, in this technique.

The most frequent criticism leveled at the thin-needle technique is the implantation of tumor cells along the needle tract. Berg and Robbins (1), in a 15-year follow-up of 370 cases of breast cancer that were aspirated and 370 matched controls, found that the long term prognosis slightly favored the aspirated cases. This would seem to indicate no unfavorable effects from the thin-needle aspiration technique. Engzell, et al. (9), have also studied aspiration experimentally in the popliteal lymph node in rabbits seeded with the Vx2 carcinoma cells. The efferent lymphatics and efferent vein were cannulated and material was collected during the aspiration with massage of the lymph node. In one case out of 16, tumor cells were found in the blood and lymph after massage and thin-needle aspiration.

The authors (9) also reported the clinical follow-up of 124 cases of benign mixed tumors after ten years and found three local recurrences at 4, 5 and 9 years. No tumor was found in the needle tract on serial sectioning. In addition, they studied 242 patients with prostate carcinoma who had been followed for five years after thin-needle aspiration. One patient of this group developed transrectal growth three years after needle aspiration. It would seem from these studies that this technique is safe from the criticism of implantation of tumor cells in the needle tract.

**Brushing Cytology.** The second area of cyto-
logic investigation has been brushing cytology using the various fiber optic scopes, particularly in the respiratory and gastrointestinal tract. In the respiratory tract, this is the outgrowth of the selective catheterization technique of Nordenstrom and Carlens (23) and the disposable bronchial brush designed by Fennessey (13, 14).

We prefer the bronchial brushing under direct vision using the fiber optic bronchoscope. This allows visualization of any lesion in the major and most of the secondary bronchi as well as the respiratory excursions. Any lesion seen may be sampled directly and the brush may be pushed into the parenchyma of most of the bronchial segments for peripheral sampling. Our experience to date, involves 39 cases with cytologic abnormalities, including 16 carcinomas mostly of the squamous type and 18 cases with various atypias of reserve cell hyperplasia, squamous metaplasia or bronchial hyperplasia. We have had four unusual cases as follows: cytomegalic inclusion disease in a transplantation case, pneumocystis carinii in a patient with myeloma, adenovirus infection and the identification of ferruginous bodies along with atypical alveolar pneumocytes.

We have had the best success in smear preparation by having a technologist available in the room where the brushing is being done to make the smears. Two or more smears are made using totally frosted Dakins slides2 which have been premoistened with ethyl alcohol. These are immediately fixed in 95% ethyl alcohol. Just because the slides are damp, cellular material is not lost and drying artifacts which occur very rapidly with bronchial epithelial cells are avoided. This is extremely important. Failure to follow this simple technique will result in poor quality smears and will discourage the physicians from using this very valuable technique. Following the making of the smears, we shake the brush in a small amount, 10 ml, or physiologic saline or Polysal®; from this material filters are prepared, either nucleopore or millipore. The material is all stained by the Papanicolaou technique. If fragments of tissue are obtained, as they occasionally are, they may be processed as a biopsy.

The results from several series are reported in Table 2 (16, 17, 18). A false positive rate has been obtained in these series of less than 1% or one case. The best results are reported by Hattori et al. (18), who attained an accuracy of 91.6% in 12 cases of lung cancer. In this series there were no false positive cases. Even in the community hospital where there might not be special interest in this technique, Funkhouser and Meininger (17) reported 70% accuracy. Combining this with bronchial and sputum exfoliative cytology, an overall accuracy of 83.8% was obtained in the diagnosis of lung cancer.

Contraindications to the technique as outlined by Janower and Land (20) are patients who are in poor general condition or who cannot tolerate any bronchial irritation. The advantages to the technique, in our opinion, are that the quality of the material is greatly improved from the cytologic point of view. Secondly, a more specific diagnosis can be rendered, particularly in inflammatory and infectious conditions. Thirdly, secondary segmental bronchi and peripheral lesions can be reached and bronchi can be selectively sampled, particularly in “early” lung cancer cases.

The technique is also very valuable in lesions of the esophagus and stomach. The Japanese have pioneered the technique through their use of fiber optics. In their hands, with the early diagnosis of gastric cancer by this method, they have reported a survival in 365 patients of 92.5% for five years (19). While this technique has not generally been used in the United States, the results reported by Prolla et al. (24), summarized in Table 2, are quite impressive. The false positive rate is very low and is better than conventional lavage cytology. The false negative rate is much lower than the conventional lavage cytology. The advantages are the simultaneous brushing with endoscopy. The cytology is selected from the lesion seen. The cytologic procedure takes only a short time in contrast to the collection, preservation and centrifugation for conventional lavage cytology. The preservation and cellularity of the material is much better and there are no complications (21). Our own experience with this technique in a limited series to date would confirm all of these findings. Particularly to be stressed is that the quality of the material cytologically is much improved.

Summary. Reviewed in this paper are two cytologic techniques, thin-needle aspiration and brushing cytology, which have added immeasurably to the diagnosis of particularly malignant disease in a variety of areas. The advantages of the techniques are that they bring the cytology closer to the source of

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2 Trident Microscope Slides-Rectangular Dakin, Aloe Scientific, St. Louis, Missouri 63103.
TABLE 2

<table>
<thead>
<tr>
<th>BRUSHING CYTOLOGY</th>
<th>RESULTS IN SELECTED SITES</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Cases</td>
<td>Correct Diagnoses</td>
</tr>
<tr>
<td>Bronchi and Lung* (16, 17, 18)</td>
<td>114</td>
</tr>
<tr>
<td>Stomach and Esophagus (24)</td>
<td>269</td>
</tr>
<tr>
<td>Carcinoma and Lymphoma (54)</td>
<td>98.6%</td>
</tr>
<tr>
<td>Benign Conditions (215)</td>
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</tbody>
</table>

* Combination of three series
† No false negatives among malignant tumors
@ One case

the lesion, particularly in the brushing cytology; the quality of the material is much improved over conventional cytologic techniques and the complications to the patient and the expense of the procedures are minimal.

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REFERENCES


