Aspiration Biopsy Cytology

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Summary
After the presentation of the technique of the aspiration biopsy and the mode of preparation and staining of the smears, the author discusses the usefulness and limitations of this procedure in the diagnosis of benign and malignant lymph node lesions.

Studies on blastocytic transformation in short-term tissue cultures in the presence of phytohaemagglutinin have indicated that the cells are unimpaired by the needling and/or the negative pressure used in the aspiration. Since the patient experiences little discomfort from fine needle biopsy, the aspiration biopsy of lymph nodes can also be used for sampling cell material in clinical research.

The problem of dissemination of tumor cells via the needle track or efferent lymph or blood vessels is also discussed. The use of fine needle aspiration biopsy in diagnosis of malignant tumors does not appear to involve a risk of tumor spread.

Introduction
Lymph nodes were possibly the first structures to be studied by aspiration biopsy. The earliest report seems to have been that published in 1904 by Greig and Gray (4). They needle lymph nodes to search for trypanosomes in sleeping sickness. In 1921 Guthrie (5) described needling of lymph nodes as a diagnostic method and cited a case of lymph node metastasis from an unknown primary tumor. Since then, aspiration biopsy of lymph nodes has been increasingly advocated; the extensive literature includes a number of monographs and comprehensive surveys in textbooks (7, 8, 10-12, 14).

The information desired from aspiration biopsy of lymph nodes depends on the history in the individual case. In patients with known primary cancer, the aim is to reveal local or distant metastases. When no cancer has been established but enlarged lymph nodes suggest malignancy, cytologic examination of biopsy aspirate may confirm this suspicion and also show whether the lesion is a malignant lymphoma or a metastasis from an occult primary tumor of some other type. Even when the tentative clinical diagnosis is benign lymphadenitis, the clinician may wish to have his interpretation confirmed cytologically.

Aspiration biopsy of lymph nodes can also be used in clinical research to study, for instance, the effect of therapy with repeated aspirations. Since the patients experience little discomfort from fine needle biopsy, the serial examinations required for observing human tumors during various forms of treatment are well tolerated (6).

Technique of Aspiration Biopsy
Apart from a syringe and a well-fitting needle, aspiration biopsy requires little equipment. The syringes used at Radiumhemmet have a special handle (Fig. 1). This permits a one-
hand grip while the biopsy is being performed. Fine needles (22 gauge, outer diameter about 0.6 mm) are recommended. They reduce the risk of trauma and minimize admixture of blood.

The skin is wiped with an antiseptic. No anesthesia is required. Preliminary nicking of the skin in order to avoid contamination of the aspirate with squamous epithelium or other cells from around the needle track is unnecessary when a fine needle is used.

When the needle has entered the node (Fig. 2a), the piston of the syringe is retracted (Fig. 2b); a vacuum is created in the system while the needle is guided in a straight line through the node. In this way material is drawn into the needle. In order to obtain sufficient material, the needle may have to be moved back and forth three or more times and possibly directed into different areas (Fig. 2c). Throughout this manipulation, negative pressure is maintained in the syringe by keeping the piston retracted.

To obtain a representative specimen, it is wise to make the path of the needle through the tissue as long as possible; this is very important in spleen. It may be mentioned here, that, when needling the spleen, local anesthesia is used. Palpable spleens should be punctured in inspiratory arrest, somewhere in the palpable part beneath the costal arch. Nonpalpable spleens may be reached through the ninth intercostal space in the midaxillary line during expiratory arrest (10). From this site of puncture, the distance to the spleen is about 5 cm, the needles used should be about 12 cm long (outer diameter about 0.7 mm).

The movement of the needle tip should be rather quick in order to avoid too rich an admixture of blood during its passage through blood vessels. The aspiration should be interrupted before the tip of the needle has left the node. If aspiration is continued until the tip has left the skin, the tiny specimen will disappear into the syringe and it is then usually lost.

The volume of aspirate is normally very small: a satisfactory aspirate should not be visible within the syringe. It consists of interstitial fluid, cells, and blood in varying proportions. Since the aspirate coagulates almost immediately, no time should be lost in blowing out the tiny drop on to a slide and preparing the smears before coagulation occurs. Cells caught in a coagulum are usually difficult to identify.

Staining

Methods of fixing and staining smears of biopsy aspirate have given rise to some controversy. In reports resulting from collaboration between clinicians and pathologists, wet fixation and staining, e.g., according to Papnicolaou or with hematoxylin-eosin, have been recommended in order to insure maximum similarity between the aspirated cells and the corresponding cells in tissue sections. The as-

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pirates dry fairly quickly, however, which results in variability in fixation and staining. Air-drying of smears of aspirate and staining according to the mentioned methods (originally devised for wet-fixed smears and histologic sections) are used in some centers (1). This procedure, however, results in poor staining and loss of microscopic detail.

Many workers, therefore, prefer to use air-drying of smears of aspirate with stains initially evolved for air-dried blood or bone-marrow smears. The methods of Wright (Wright-Giemsa stain) or Pappenheim (May-Grünwald-Giemsa [MGG] stain) are used as standard techniques by most hematologists. At Radiumhemmet it is customary to aspirate two or three specimens from the lesion under study. One or two air-dried smears are then stained with MGG. The smear from the remaining aspirate is fixed in 95% methanol and stained with Papanicolaou or hematoxylin-eosin. The availability of both types of smear has been found to increase the diagnostic accuracy. The wet-fixed smears give the better presentation of nuclear detail, and in the air-dried hematologic smears, there is more differentiated staining of the cytoplasm and of extracellular substances, i.e., mucus, colloid, etc.

Nuclear detail is of paramount importance in exfoliative cytology if the recognition of a carcinoma depends on the appearance of a few exfoliated cells. In aspiration biopsy cytology, other problems are encountered when smears are used which often contain hundreds

Fig. 3a and b Needle aspirates in lymphadenitis. Papanicolaou stain.
a) Lymphocytes and a few lymphoblasts (larger, pale-stained cells). x 310.
b) A syncytium of pale-stained, oval-shaped histiocytes or reticulum cells surrounded by lymphocytic cells. x 310.
of unmistakable carcinoma cells. These problems include typing of carcinomas, recognition of grades of differentiation and, when metastases are needled, establishing the site of the primary tumor. To solve these problems, the cytoplasmic details and the staining properties of cellular products as brought out by hematologic stains are of decisive value. This accounts for the usefulness of hematologic smears as complements to wetfixed smears.

**Clinical Application**

In evaluating the reliability of aspiration biopsy cytology in lymphnodes, the nature of the required information must be taken into account. In oncology the method is mainly used to obtain microscopic confirmation of a clinical diagnosis of lymphadenitis (Fig. 3), malignant lymphoma (Figs. 4, 5, 6) or metastatic carcinoma (Fig. 7).

Cytologic confirmation of a *clinically benign* lesion is fairly simple. If the following four basic rules are observed, the method is fully reliable. These rules are:

1. If two or more lymph nodes are palpable, at least two should be needled.
2. The results of a complete hematologic examination for the case under study should be reported to the examining cytopathologist.
3. When the cell pattern is monotonously lymphocytic, the node must be extirpated for histologic study if the clinical picture does not improve.
4. When any cellular atypia is seen in an otherwise normal smear, the needling must be repeated after 2 to 4 weeks if the palpatory findings do not normalize.

The problem is usually much more complicated when the clinician requests aspiration biopsy of a suspected malignant lymph node. If the cell population has a monotonous, but benign appearance; histologic examination should always be done since about 20% of cases of differentiated lymphocytic lymphoma cannot at present be diagnosed with certainty in smears of aspirate. Repeat needling in these cases contributes little to the definitive diagnosis. When cellular atypia is present, and the cytologic diagnosis is well-differentiated lymphoma, this diagnosis should be confirmed by histologic examination of the needled node if the malignancy does not appear to be advanced. In advanced cases the cytologic diagnosis can be substantiated by the sternal biopsy which is routine for malignant lymphoma. A reliable cytologic diagnosis of poorly differentiated lymphocytic lymphoma, stem cell lymphoma, and histiocytic lymphoma can be made by an experienced examiner.

In Hodgkin’s disease the finding of frank Reed-Sternberg cells (Fig. 6), in smears of biopsy aspirate is a clear diagnostic sign. The initial aspiration biopsy, however, gives a definite diagnosis in only about 70% of cases. When the cytologic findings are inconclusive and there are multiple enlarged nodes, the node with the most suspect appearance should...
be extirpated for histologic study. If there is a single enlarged node and the clinical and cytologic findings are uncertain (e.g., the cytologic examination suggests granulomatous lymphadenitis), it is usually advisable to repeat the aspiration after 2 to 4 weeks. If the clinical and cytologic pictures have not then clarified, the lesion should be extirpated and histologically examined.

These recommendations are based on the experience that Hodgkin's disease in its earliest stages is also difficult to diagnose histologically. Excision of a single lymph node followed by an inconclusive histologic report is of little value to either the clinician or the patient.

The diagnosis of carcinoma from lymph node aspirate is dependent on the clinical judgement that the needled structure was, in fact, a lymph node. Apart from those rare instances when lymphatic cells are aspirated together with cancer cells, the cytologist who observes cancer cells should, strictly speaking, report only that cancer cells are present in a structure that clinically appears to be a lymph node.

The accuracy of cytologic diagnosis from lymph node aspirate was evaluated by comparing the cytologic reports and the subsequent histologic findings in 257 histologically confirmed cervical lymph node metastases. In 232 cases (90.3%) the cytologic findings was carcinoma; in 8 cases, (3.1%) suspected carcinoma; and in 17 cases, (6.6%) benign lesions (3). The explanation of the negative reports may have been either that, at the time of needling, the metastasis involved only a small part of the node and was missed by the needle, or that the metastasis arose in the interval between needling and extirpation of the node. This interval ranged from a few days up to 12 months. Although repetition of the aspiration biopsy in clinically suspected but cytologically negative cases may be expected to reduce the number of false negative cytologic reports, repeatedly negative findings should not unreservedly be considered to exclude malignancy. If any clinical doubt persists, the lymph node should be excised and histologically examined.

Biological Studies

The use of aspiration biopsy for biologic studies of cells (Fig. 8) necessitates the consideration of possible effects of the aspiration procedure on the viability of the sampled cells. When pressure differences similar to those created during aspiration biopsy were applied in vitro to HeLa and bone marrow cells in suspension (13), there was no apparent deterioration of cell viability or proliferation. This suggested that the negative pressures used in aspiration biopsy do not per se affect the viability of cells drawn into the needle.

The viability of cells collected by aspiration biopsy from the spleen, lymph nodes and bone marrow of living animals was studied (9). Various negative pressures were used during the aspiration, and cell viability was assessed by dye permeability and trypsin digestion tests. The viability of cells aspirated from human lymph node affected by various pathologic conditions was evaluated by studying blastocytic transformation in short-term tissue cultures in the presence of phytohemagglutinin (PHA) (14).
When the negative pressure during the aspiration was about 100 mmHg, the mean score of viable cells was about 80%. Increasing this pressure from 100 to 700 mmHg had only a slight effect on the percentage of viable cells in the aspirate. These results suggest that some damage occurred to the cells during their detachment from the surrounding tissues by the needling and/or the negative pressure. Once within the needle, the prevailing pressure apparently had no effect on cell viability. The blastocytic transformation that occurred in the PHA tests on lymphocytes aspirated from human lymph nodes (Fig. 9) indicated that the cells were unimpaired by the needling and the negative pressure used in the aspiration.

**Complications**

Dissemination of tumor cells or cell clusters via the needle track or efferent lymph or blood vessels with resultant extension of the neoplastic growth and impaired prognosis is a conceivable risk in aspiration biopsy. However, most malignant tumors (needling sites included) undergo therapeutic measures such as surgical excision or irradiation after aspiration biopsy; this minimizes the risk of tumor spread via the needle track. At Karolinska Sjukhuset, 656 patients in whom cervical lymph node metastases had been diagnosed by aspiration with a 22-gauge needle were followed up for five years. In no case was clinical evidence of local tumor growth resulting from the aspiration biopsy present.

The literature contains numerous reports on the spread of tumor cells via blood vessels in connection with surgery. Much less attention has been paid to the possibility of vascular or lymphatic spread in connection with aspiration biopsy. The question nevertheless merits serious consideration since no matter how fine the needle is, aspiration biopsy inevitably causes microtrauma to the tissues through which the needle passes.

In an experimental approach, efferent lymphatics and veins in rabbits with popliteal lymph node metastases from V<sub>x</sub>2 carcinoma were cannulated (2). Efferent lymph and blood were then sampled and analyzed for tumor cells. After gentle massage of the metastasis-bearing node, carcinoma cells were demonstrated in the lymph in one of seven rabbits and in the blood in one of nine rabbits. The node was then needled. No evidence was obtained that this needling released V<sub>x</sub>2 carcinoma cells or cell clusters into the lymphatic or blood circulation.

The available data thus indicate that, although it is possible for tumor cells to enter the needle tracks in aspiration biopsy, this route of possible dissemination lacks practical clinical implications. Distant dissemination of tumor cells through lymphatics or blood vessels following fine-needle biopsy seems to be uncommon judging from the cited experiments on rabbits. The use of fine-needle aspiration biopsy for diagnosing malignant tumors, therefore, should not involve a risk of impaired prognosis.
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