1 INTRODUCTION TO FINE NEEDLE AND CORE BIOPSY: TECHNIQUES

INTRODUCTION

This text attempts to provide an approach to the diagnosis of the protean diseases that affect lymph nodes and spleen through the combined use of fine needle biopsy (FNB) and core biopsy, and the optimal use of small biopsy material for ancillary tests. The word "cytohistology" refers to this use of both histologic and cytologic techniques to assess small biopsy material and also to the fact that cytologic findings are best understood and interpreted by correlating with the associated histopathology. The fine needle biopsy and core biopsy are complementary synergistic tests.

Fine needle biopsy provides a rapid, cost effective, safe, patient friendly and accurate method of assessing localized and generalized lymphadenopathy, two of the commonest clinical presentations to general and specialist hospital-based medicine. This applies worldwide in both highly developed and resource-limited medical settings, but the role of FNB is enhanced by the availability of image guidance for sample acquisition and the concurrent use of ancillary tests including cell blocks, cultures, immunohistochemistry (IHC), flow cytometry, cytogenetics, and *in situ* hybridization. Core biopsies are complementary in that they offer a larger volume of material for histological assessment and facilitate the use of extensive IHC, especially in cases of lymphoma and metastatic malignancy. The FNB with or without core biopsy is almost always done as an outpatient or day surgery procedure, which greatly reduces the need for open surgical lymph node excision biopsy with its frequent need for a general anesthetic, operating theatre time and space, greater cost, and at least some, and often significant, scarring.

The indications for the use of FNB in the diagnosis of lymph node lesions are the investigation of palpable peripheral single site or generalized lymphadenopathy and impalpable deep-seated lymphadenopathy, where the differential diagnosis (DD) includes in children and young adults specific infections, nonspecific reactive lymphadenopathy, metastatic small round cell tumors, leukemia and lymphomas, most commonly lymphoblastic lymphoma, and in adults, specific infections, reactive lymphadenopathies of various causes but often nonspecific, metastatic carcinomas and lymphomas.

The FNB in our view nearly always should be the first diagnostic test performed for both peripheral palpable lymphadenopathy and deep-seated lesions accessible by endoscopic, endobronchial or percutaneous FNB. This is dependent on having personnel skilled in the FNB technique and interpretation, but is the most cost-effective initial test. In this context FNB is a powerful triage tool that can confirm that the lesion is a lymph node, and then can categorize the cause of lymphadenopathy into nonspecific reactive processes, specific infections such as tuberculosis, metastatic disease, or lymphoma.

Once the FNB material is available and the more common causes of lymphadenopathy have been excluded, it is sensible, cost effective, and beneficial to both the patient and the health care system to take the opportunity to use the FNB and concurrent core material when available, for the diagnostic workup of lymphoma. The primary diagnosis of lymphoma can be made in many cases depending on the type of lymphoma and on the expertise of the cytopathologist and availability of ancillary tests, including flow cytometry and molecular tests, although we recognize this is still contentious in many centers. The diagnosis of recurrent or residual lymphoma can be made to the satisfaction of most hemato-oncologists by cytology alone with the use of appropriate ancillary tests when required.

In review articles of the literature in the era before the routine use of IHC and flow cytometry, the diagnostic accuracy in diagnosing lymphomas and classifying them according to the REAL or other classifications is usually quoted in the range between 60 and 80%. In specialist units in the current era using cytomorphology and the full range of ancillary tests, this accuracy is up to 95%. The current WHO classification of lymphoma is more complex

with many new entities, but it emphasizes clinical and cytomorphological features and the use of immunophenotyping and cytogenetics rather than the nodal architectural features which were relied on in previous lymphoma classifications, making it more suitable for diagnosis by FNB and ancillary testing. Diagnosis of Hodgkin lymphoma (HL) is possible in up to 85% of cases, but subclassification requires core or, in many institutions, reflex excision biopsy prior to treatment. Flow cytometry can exclude B-cell lymphomas but cannot make the diagnosis of HL.

Many of the criticisms of the use of FNB to diagnose lymphomas definitively are based on studies which suffer from a small number of cases, a variability of case mix related to primary diagnosis of lymphoma versus recurrence, a lack of followup, a lack of uniformity in the experience and skill of the FNB operator and pathologist reading the smears, and the patchy availability and use of ancillary testing. Series which involve FNB performed by practitioners who rarely perform lymph node FNB or prepare smears, or do not use available ancillary tests, do not reflect the current best practice of FNB in established institutions where well trained operators perform and interpret FNB, with or without the benefit of ancillary testing, which, of course, is recommended but not readily available in developing countries.This issue is discussed more fully in Chapter 4.

An important part of the evaluation of the FNB is an assessment of the extent to which it provides a complete diagnosis on which treatment decisions can be based. This assessment should be reflected in the report, which should also include recommendations for additional investigations that might include repeat FNB, cores or excisional biopsy.

If core biopsy or excisional biopsy is considered necessary, the triaging FNB in most cases will have excluded infection or metastatic disease, and the surgical specimen can be handled most effectively to maximize its use for immunophenotyping and molecular and cytogenetic studies including *in situ* hybridization and polymerase chain reaction (PCR). Where general anesthesia is used for neonates and children and other patients unable to cooperate with a FNB, it can be efficiently combined with other procedures such as bone marrow biopsy or in some cases biopsy of spleen or liver. In assessment of recurrent lymphoma, FNB with flow cytometry can exclude opportunistic infections in enlarged lymph nodes by cytology and cultures, and diagnose the lymphoma if present or if changed in subtype in most cases.

TECHNIQUE: PERFORMING THE FNB AND MAKING SMEARS

What to do before performing the FNB

The FNB should be part of a "triple test" which includes clinical assessment, imaging where necessary, and the FNB. Ideally, the FNB is performed by an experienced cytopathologist or radiologist skilled in FNB technique, who has the clinical information and radiological findings to help plan the FNB and selection of ancillary tests.

A detailed clinical assessment including directed history and physical examination should precede FNB of peripheral and deep-seated lymph nodes. A full blood count is extremely useful when a lymphoproliferative or myeloproliferative disorder is high on the differential diagnosis. Any history related to a bleeding diathesis or complications related to previous procedures, such as vasovagal syncope, should be recorded. Appropriate imaging including chest X-ray and ultrasound of the involved lymph nodes should be obtained where available. However, the extent of radiologic investigation preceding FNB should be guided by the clinical assessment. In an otherwise healthy patient, ultrasound examination of a circumscribed subcutaneous lump prior to FNB is not necessary and may be misleading. A variety of lesions such as epidermal cysts and salivary gland tumors that are easily diagnosed by FNB are often misinterpreted as lymph nodes on an initial ultrasound.

The clinician performing the FNB should have formed a DD for the cause of the lymphadenopathy based on the age of the patient, the site of the lymphadenopathy and the overall history, the clinical presentation, physical findings, and imaging prior to performing the FNB, so that the appropriate ancillary tests can be anticipated prior to rapid on-site evaluation (ROSE). A painful lymph node rapidly enlarging over days to one or two weeks, associated with local tenderness and perhaps an obvious skin lesion, will have infection as the probable cause, but painless "cold" lymph nodes can also be caused by infections as in cervical lymph nodes in children with mycobacterial infections, and relatively quickly enlarging nodes can be related to necrosis in metastatic carcinomas and melanomas, lymph node infarction, and high-grade aggressive lymphomas.

Slowly enlarging, usually painless, lymph nodes, especially those over 2cm and generalized or deep-seated in the retroperitoneum and thorax in patients over 40 years, are much more likely to be malignant. However, sarcoidosis and tuberculosis can cause significant mediastinal and

peribronchiolar lymphadenopathy, and less commonly retroperitoneal enlargement of lymph nodes; and less common diseases such as Rosai–Dorfman and Kikuchi's disease can cause peripheral, usually cervical, lymphadenopathy.

Most cases of peripheral palpable lymphadenopathy in children and young adults will be reactive and the cause will be related to socioeconomic and geographic factors. In developing countries the most common causes will include mycobacterial infections, while in developed countries the commonest cause will be nonspecific infections and reactive processes, where the etiology despite cultures and serology will remain uncertain, but is caused most likely by viral and bacterial infections. Other specific infectious causes that can be diagnosed on FNB will include pyogenic bacterial infections, infectious mononucleosis, toxoplasmosis, fungal infections, and cat scratch disease. The distinction of reactive processes from malignant lymphoma and HL remains the most problematic area. Flow cytometry has had a major impact on the diagnostic accuracy of FNB material in these cases.

It is vitally important to check for any history of previous malignancy including lymphoma, and determine if the patient is immune compromised.

Performing the FNB

Before FNB is performed the patient should give informed consent and this should be recorded according to institutional protocols. Some institutions treat superficial FNB as analogous in risk to phlebotomy, and do not require a signed consent form, which is reserved for deep-seated lesions, while other institutions require a signed consent for all procedures. Whatever the policy, the patient should be informed not only of the potential degree of discomfort of the procedure, the number of needles necessary, and the small risk of complications, but also of the likelihood of a diagnosis being achieved and the possibility of additional procedures being necessary.

In an ideal situation, the FNB operator should have a clear DD based on the clinical and imaging findings and follow a set protocol with one consistent technique that recognizes that all FNB and core biopsies have potential infection risks to the operator. This allows the operator to maximize the diagnostic usefulness of the FNB and core biopsies for the individual patient's care. Ideally, the clinician performing the FNB is a cytopathologist who will report on the FNB. In situations where radiologists or oncologists perform the biopsy, then having a cytologist or cytopathologist on site is of great benefit, because they can expertly carry out the smearing of the cytology slides, provide ROSE for adequacy of the material, request further passes for special studies, request core biopsy, and triage the case so that ancillary studies can be most efficiently and cost-effectively ordered.

The FNB is inherently a simple procedure but requires considerable practice to be consistently successful. No anesthetic is required although anesthetic patches may be of use in children. Simple alcohol swab preparation of the skin is all that is needed. There is no reported risk of spreading infection or seeding of tumor from FNB of palpable or impalpable lymph nodes. There is a recent report of spread of metastatic melanoma from a periesophageal lymph node into the esophageal wall, which may have occurred without the FNB. Other complication risks of necrosis or infarction of the lymph node following FNB are rare.

The key to good quality FNB of lymph nodes is to recognize that it is the repeated, rapid passaging of the fine needle itself into and just through the lymph node that gains good material, and not aspiration via a syringe holder or other method. This is a FNB not a "fine needle aspiration biopsy." A nonaspirating technique simply using a 22 to 27 gauge needle held between thumb and index finger, rapidly passed into and out of the node in a "woodpecker" fashion, while twirling the needle, yields excellent results. After ten to 15 passages into the node or as soon as any material or blood is seen in the hub of the needle, the needle is withdrawn and gentle pressure applied to the FNB site. The needle-only technique gives the operator excellent control of the needle and fine sensation of the needle as it passes through the nodal capsule into the node substance. This delicate sensation can be felt and assists in judging how much force should be used to push the needle into a soft reactive or lymphomatous node or a firm fibrotic node involved by nodular sclerosing Hodgkin lymphoma (HL) (Figure 1.1 A,B).

Aspiration, especially when applied early in the FNB process, increases blood contamination of the lymphoid or other material in the lymph node. Excess blood dilutes the lymphoid material and distorts the lymphoid cell shape by affecting fixation, while the red cells impinge on the cytoplasm and cause poor nuclear morphology and staining that precludes assessment of cell size and nuclear features. However, the use of a fine needle attached to a syringe in a syringe holder is also a good technique when the cutting action of the needle is used primarily, and aspiration only applied late in the lymph node FNB.

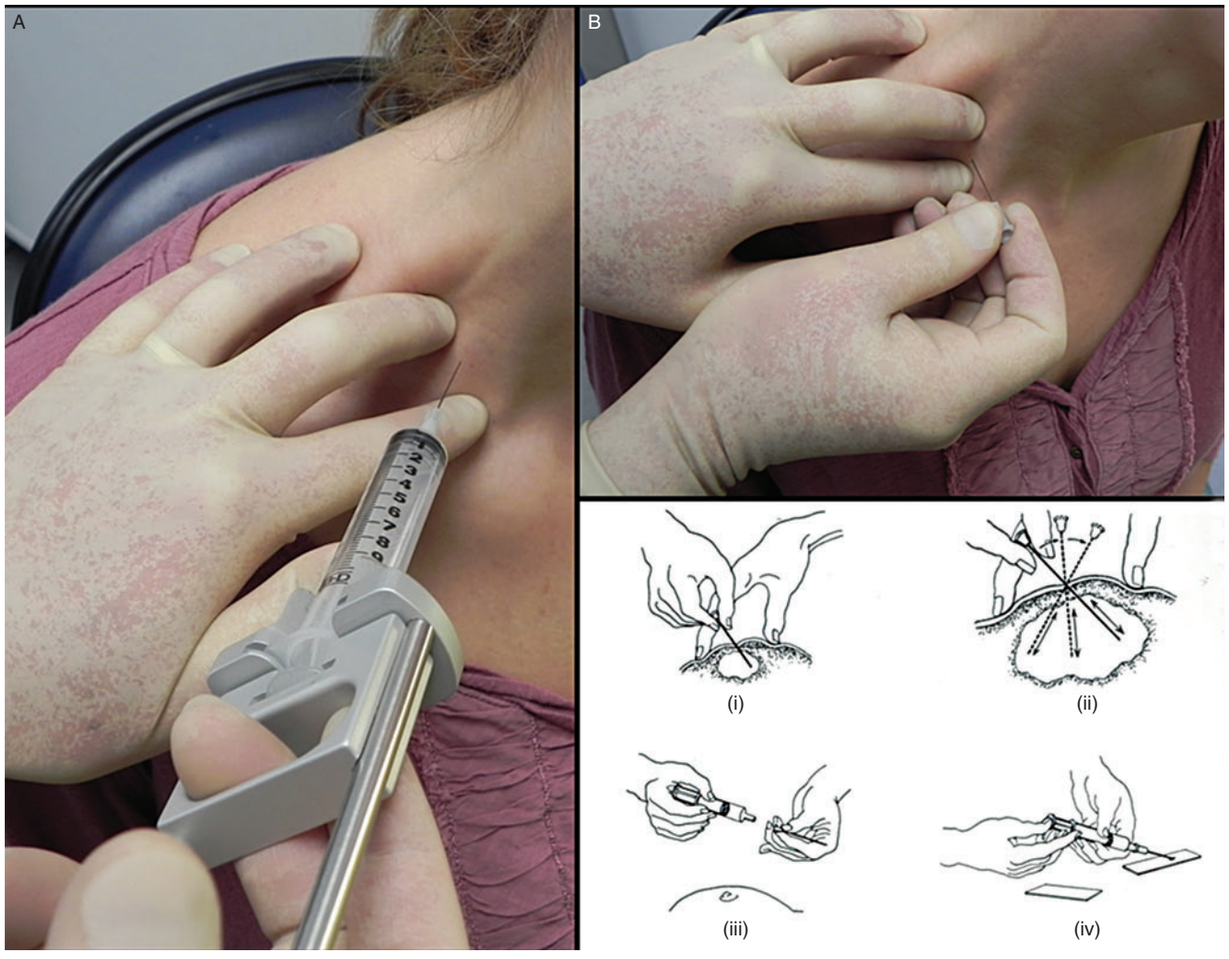

Figure 1.1 (A) Fine needle biopsy with negative pressure applied late in the procedure using a Cameco® syringe pistol. (B) In most cases the pipette or "Zajdela" technique yields cellular samples without any suction. (i) Hand position. The tumor is immoblized with one hand. The fine needle is introduced into the tumor with the other hand. (ii) The needle is moved back and forth very slightly as it is angled in different depths of the tumor before it is withdrawn. (iii) The needle is removed and connected to a syringe filled with air. (iv) The cellular material is expelled onto a glass side. Technique as illustrated in Zajdela's paper (Zajdela A, Zillhardt P, Voillemot N. Cytological diagnosis by fine needle sampling without aspiration. *Cancer* 1987; **59**(6):1201–5).

Image guidance of the FNB theoretically offers advantages in sampling the lymph node. However, it has inherent problems. In cases where the FNB is done under image guidance, it is essential not to leave the needle in the node for too long a period while confirmatory ultrasound images are taken to prove the needle was in the lesion, as clotting will occur in the needle. Fibrin clot will form and damage and obscure the lymphoid material, prevent good smearing and eventually form coils of clot deposited on the slide which contain most or all of the lymphoid material, masking and preventing diagnosis.

Different angles of approach for each FNB pass are recommended to maximize sampling of the lymph node. This is more easily achieved when the FNB is done by palpation, than when the angle of approach is determined by the ability of an ultrasound probe to locate and maintain an ultrasound image of the FNB needle and the lymph node simultaneously. When the needle is deep within the lymph node, the angle of the needle must not be changed, as this moves the tip of the needle through a cutting arc within the node. The needle should be withdrawn almost to the skin, and then the angle changed if this is desired in the one FNB pass.

Sterile gloves are worn, and if HIV infection is suspected or the FNB is occurring in an area where HIV is endemic, double-gloving of the nonoperator hand that is palpating and fixing the lesion is recommended. The finger tips of the palpating hand should fix the lymph node as far as possible, "lifting" the node towards the needle, taking care not to place the finger tips deep to or behind the needle tip. The flat of the hand or fingers rarely helps to fix the palpable node. If ultrasound is being used to direct the FNB, then

fixing the node should be attempted using one hand of the operator or an assistant, while the ultrasound probe assists in pressing onto the node.

When the syringe holder is being used by a right-handed operator, and held in the right hand, all movements to fix or remove the needle from the syringe by the left hand should start at the base of the syringe away from the point of the needle, with the left hand sliding down the syringe barrel to the needle, and never bringing the left hand towards the point of the needle. After each needle pass, the needle is removed using this left hand "down the barrel" technique, air sucked into the syringe, and then material expelled onto the glass slide. An RPMI or normal saline is then sucked up through the needle into the syringe, and this then returned to a correctly labeled jar for flow cytometry or cell block preparation, as required. The needle is then removed using this technique and deposited in a sharps container. It is not left lying on the work area unless in a set place away from the operator. This aspiration technique using syringe and syringe holder can be used when it is not certain the lesion undergoing FNB is a lymph node, particularly if it could be cystic, or where a "closed system" is preferred in suspected infectious cases, or where more material is sought for culture, flow cytometry or cell block preparation, because blood contamination is not a major issue in these samples.

Glasses, gowns, and face masks can be worn when performing FNB on patients with known infections on palpable or deep-seated lesions, to minimize conjunctival and mucosal splash injuries. These procedures should occur in designated rooms, rather than corridors or busy wards, although FNB can be performed in operating rooms, accident and emergency, and intensive care situations with appropriate care. If the material has clotted in the needle, care should be taken with forceful expelling of the material onto glass slides and it is recommended that in these cases, the material should be placed into saline for cell block preparation. Complicated techniques of transferring bloody material from one slide to another are not recommended, and again it is simpler to place excess or bloody material in a cell block.

In most FNB of lymph node protocols, but particularly if the smears are bloody and ROSE is not available, a minimum of two and preferably three FNB passes are made to provide Giemsa and Papanicolaou stained smears, as well as material for a cell block for IHC, immunophenotyping by flow cytometry, and culture. Once the FNB smears are assessed in the laboratory, decisions can be made regarding the use of the various rinsings. There is no point doing flow cytometry if the smears show metastatic carcinoma.

Making FNB smears

Making FNB direct smears as with the FNB itself, appears easy but is a similarly crucial step in the whole process. If the FNB is not performed with skill and the smears not made with care then the whole FNB will be a waste of time for patient and clinician.

The material from each pass is split onto at least two slides, one air-dried for Giemsa staining and one immediately fixed in alcohol for Papanicolaou staining, using the following method (Figures 1.2A and B):

1. The material is expelled gently from the needle using air in the reattached syringe, holding the needle at a right angle and just touching the slide, at a point about two-fifths of the slide length from the label end.
2. A spreader slide is picked up, and held face down at a right angle to the original slide, just touching on its leading edge the original slide. The spreader is then gently hinged down onto the material till material is picked up on the spreader.
3. The spreader is then moved along, away from its label, and gently lowered onto the remaining material on the original slide, and a smear made and immediately dropped into 100% alcohol or modified Carnoys alcohol fixative in a jar. This has to be done in a split second to avoid air drying artifact to this slide.
4. The spreader with the originally picked up material is then applied to a new slide and a second smear made. This is air-dried as quickly as possible and, in a clinic situation, a simple cheap hairdryer on low setting held 10 cm from the slide is recommended to speed the process and prevent slow-drying artifact. The use of a hairdryer in operating or endoscopic ultrasound (EUS) or endobronchial ultrasound (EBUS) theatres should be cleared as safe before usage. The spreader usually has limited material on it.
5. The spreader can be lowered or hinged onto the deposit on the original slide three or four times rather than twice, and three or four slides then smeared, or one of the lifted blobs of material on the spreader can be transferred to another slide.
6. This new slide can itself be touched by a new spreader to multiply the number of slides prepared for special stains such as the Ziehl–Neelsen.

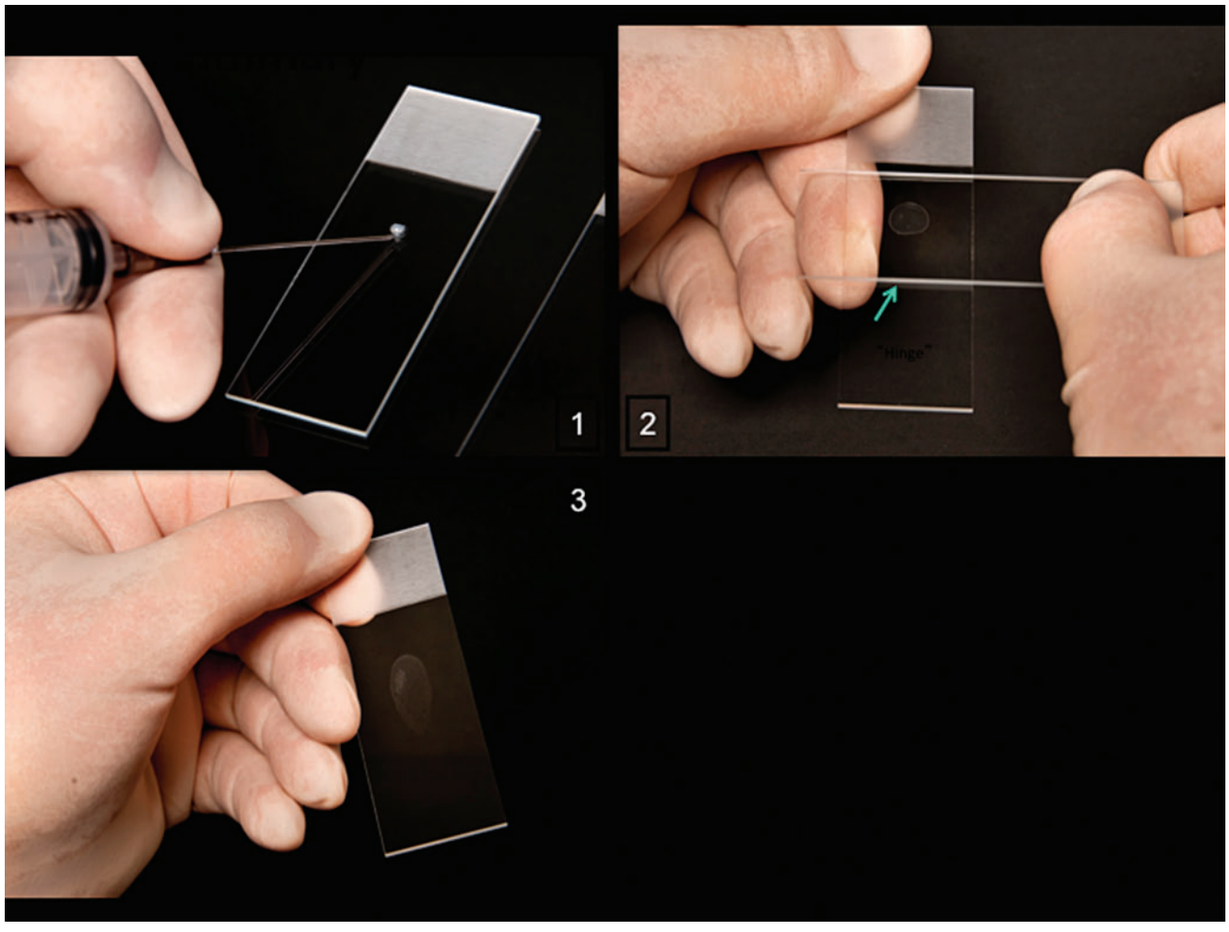

Figure 1.2A The "onestep" technique: 1. Deposit sample near label end of slide – remembering to control the hub of the needle – and place bevel of needle down. 2. Gently lower one edge of a clean slide, the "spreader," with label or "face" down, onto the sample-bearing slide, and gently "hinge" (arrow) the spreader down, allowing the material to spread by capillary action. 3. Pull the top "spreader slide" down the length of the lower slide, smearing the material, keeping the two slides absolutely parallel, and producing the characteristic oval smear. Do not "rock" the spreader.

Common pitfalls (Figure 1.3) are:

1. Expelling the material in the needle with too much force.
2. Depositing the material towards the bottom of the original slide, making smearing difficult within the space available at the tail of the slide.
3. Rocking the spreader, creating lines of crush artifact and thick material and often depositing the material on the edge of the slide, where it will be thick and not covered by the cover slip.
4. Applying too much pressure, crushing the fragile lymphoid or other cells, or applying too little pressure and leaving the smear too thick for assessment. When gently hinging the spreader onto the material, an experienced operator can sense the nature of the material. Lymphoid material tends to be soft and allows for smooth smearing, similar to pus, while metastatic carcinomas, nodular sclerosing HL and mycobacterial infections tend to be grittier on smearing, apart from necrotic squamous cell carcinoma and mucinous adenocarcinomas, which are slippery. These macroscopic features should alert the operator to the diagnosis, supported by ROSE, and help determine the need for extra passes for various ancillary tests.

Staining the FNB lymph node smears

Wherever possible air-dried slides for Giemsa-type stains and alcohol fixed slides for Papanicolaou (Pap) or in rare cases hematoxylin and eosin (H&E) stains should be

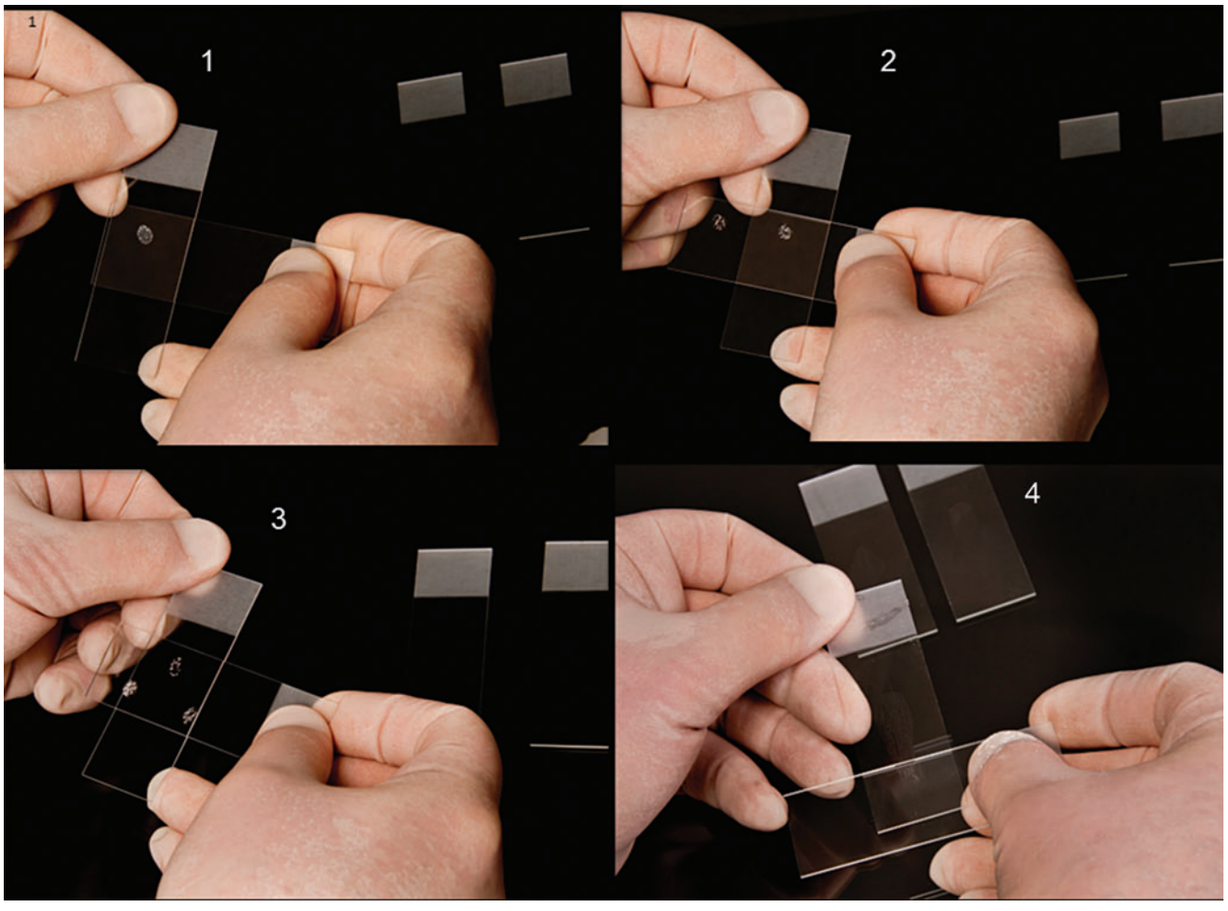

Figure 1.2B Production of multiple smears: 1. Deposit sample near label end of slide. 2. The spreader slide is hinged down onto the sample slide until it just touches and is then pulled back. 3. The spreader slide is shifted to the left and hinged down onto the sample. There are now two small drops of sample on the undersurface of the smearing slide. 4. The smearing slide is flipped over, so that the clean undersurface is used to smear the material on the original sample slide, which ideally is immediately placed in 100% alcohol for Pap stain, then flipped over again and the remaining two dots of material are smeared onto two clean labeled slides. Three slides have been prepared.

prepared. Liquid-based preparations are not encouraged because of the lack of Giemsa stains and because of the lack of all the diagnostic features of direct smears in Giemsa- and Pap-stained smears, that form the basis of pattern recognition used throughout this text.

The Giemsa smears highlight cytoplasmic features in lymphoid cells, histiocytes and epithelioid granulomas, background material including caseous necrosis and mucin, fragments of lymphoid cell cytoplasm ("lymphoglandular bodies"), which are the hallmark of lymphoid material in direct smears, yeasts and fungal elements, and the characteristic lymphoid nuclear and other features relied on by hematologists in blood and bone marrow smears. Pap smears highlight cell features that mirror the H&E stained paraffin-embedded tissue used by hematopathologists, particularly chromatin, nuclear membrane, nuclear shape, nucleoli and tingible-body macrophages. The stains are complementary and ideally should be used in all cases.

If infection is considered a possibility, then extra air-dried slides for Gömöri–Grocott methenamine silver (GMS) for fungal infections and Ziehl–Neelsen (ZN) and auramine stains for mycobacteria, and alcohol-fixed slides for Gram stain for bacteria and mucicarmine stain for cryptococcus should be made at the bedside at the time of the FNB. Also, an extra pass or at least the rinsings of needle and syringe in 2 to 3 mls of normal saline should be placed in a sterile correctly labeled container for culture. In general terms the more material available for bacteriology, the better the culture results, so a separate pass dedicated for culture is

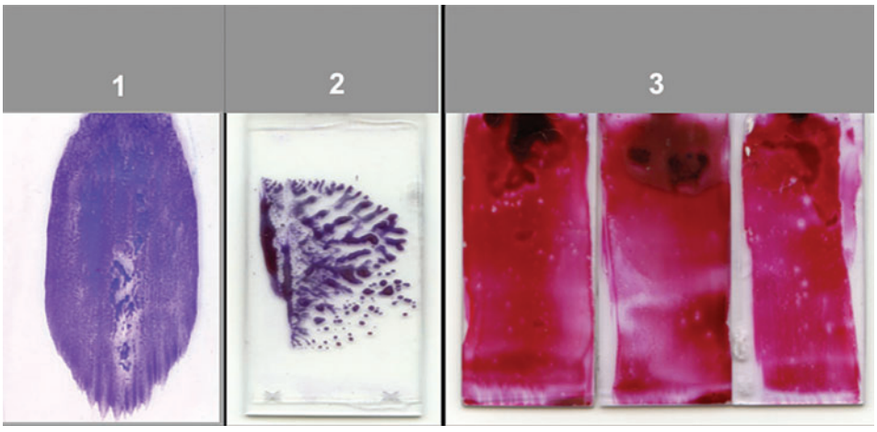

Figure 1.3 1. A well-crafted smear with bullet shape and a feather edge. In general, tissue fragments remain central and dissociated cells are distributed towards the periphery. 2. A smear made by compressing slides together and then pulling them apart. Cell morphology will be obscured in thick areas. 3. Smears with excessive blood. Cellular material is obscured by red cells and the slides are difficult to screen.

recommended. If mycobacterial infection is considered, then placing the material immediately into a suitable growth-medium container is preferable, in addition to the slide prepared for acid fast stain.

Needle rinses in saline, RPMI or Hanks medium, or preferably a whole separate pass rinsed directly into a sterile container, should be set aside for flow cytometry or cell block preparation in all FNB of lymph nodes. The cell block can be used for special stains for infective agents, IHC, fluorescent *in situ* hybridization (FISH), PCR and even electron microscopy (see Chapter 2).

RAPID ON-SITE EVALUATION

It is well established that ROSE decreases FNB inadequacy rates, increases sensitivity and specificity and overall accuracy rates, and achieves cost savings related to patient recall and use of facilities for repeat or followup procedures such as core biopsy. Material from the needle is deposited on slides with specimen splitting as described above, and the air-dried slides are express dried with a hair dryer, fixed in methanol for at least 1 minute, and then rapidly stained using a Giemsa-type stain. They can be viewed without cover slip, or they can be rapidly dried and then cover slipped. The alcohol-fixed slides prepared at the FNB can be stained later in the laboratory and a final report issued after all slides have been assessed.

At ROSE the primary aim is to assess the air-dried Giemsa-stained smears to establish that adequate material has been accessed through the FNB passes, and to request more FNB or a core biopsy if there is inadequate material. The secondary aim is to triage the material, providing a working provisional diagnosis of the lesion. Further FNB passes or core biopsy when the cytologist recognizes a lesion that will need histological material, can be requested at the bedside, facilitating the most cost effective and rational selection of ancillary testing. If ROSE is not available, the needle and syringe should be rinsed in sterile normal saline and a separate pass rinsed into saline or RPMI so that assessment at the laboratory can direct material to the microbiology laboratory for cultures, or flow cytometry for lymphoma diagnosis, or cell block preparation for immunohistochemistry for the workup of metastatic carcinoma. If the clinical workup had suggested lymphoma, then core biopsy should be performed if possible, especially in deep-seated lesions. There is no point in performing a FNB that fails to take material for these tests if they are readily available. Nor should a desultory FNB be performed because the operator is more committed to the following core biopsy. Both FNB and core biopsy should be performed as if the other modality was not available or possible to maximize the diagnostic yield of each test.

THE "NONSPECIFIC REACTIVE" FNB REPORT

In cases where the diagnosis on FNB is a nonspecific reactive process and this is consistent with the clinical and imaging workup or simply with the clinical assessment in resource-limited working environments, then it is appropriate to add in the cytopathology report a recommendation for clinical followup with repeat FNB and flow cytometry in four to six

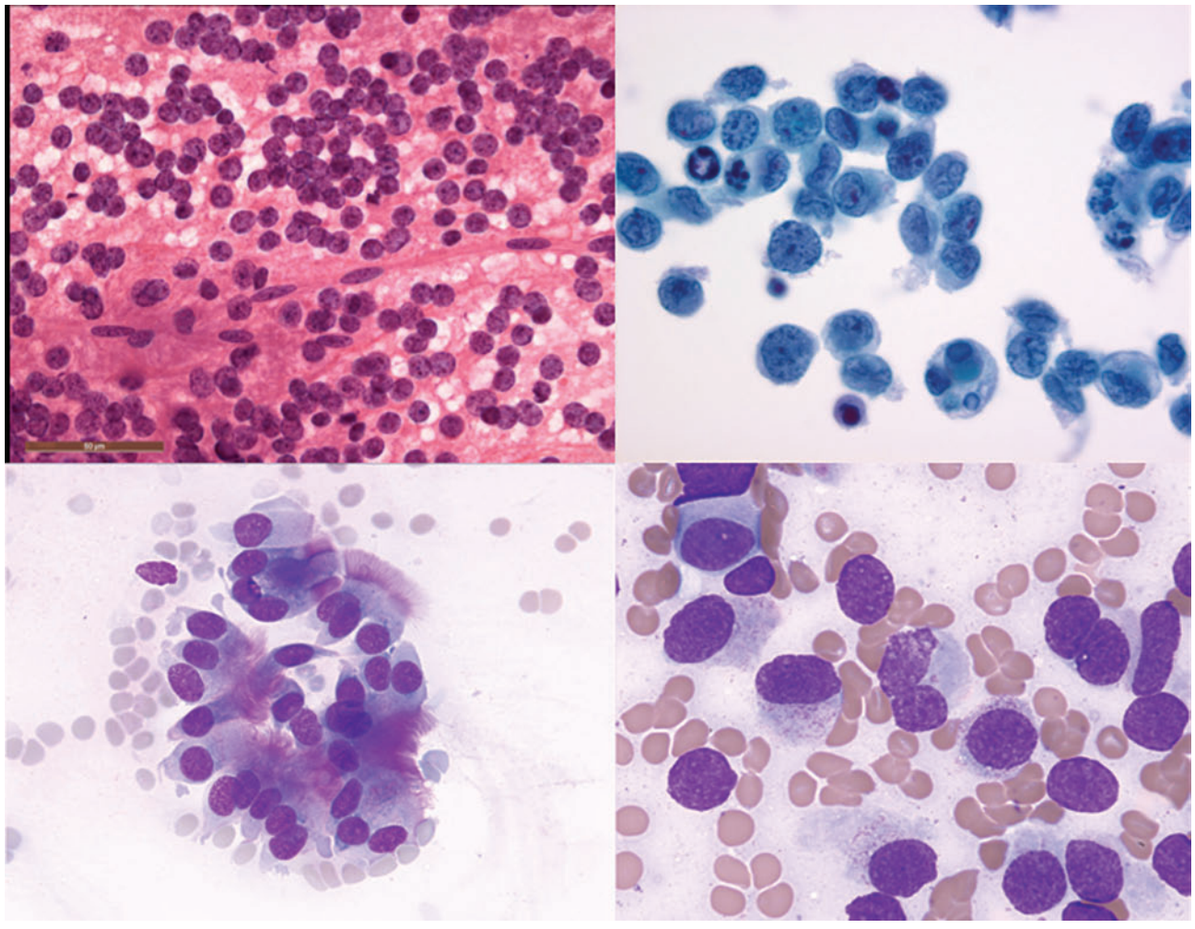

Figure 1.4 Stains. Top left: Hematoxylin and eosin (H&E) stain – smear of central neurocytoma made at the time of craniotomy, wet fixed and stained with the same H&E used for frozen section slides. Top right: Papanicolaou stain – smear of metastatic rhabdomyosarcoma. The Pap stain provides great clarity of nuclear detail and chromatin pattern. Lower left: Field's stain – contaminating benign bronchial cells obtained at EBUS TBNA of a mediastinal node. All rapid Giemsa-type stains are based on concentrated, buffered solutions of eosin and Giemsa stain. These stains can give excellent results but tend to overstain. Lower right: May Grünwald Giemsa stain – metastatic carcinoid tumor. Slower two-part Giemsa methods such as MGG are usually more consistent and provide better staining of cytoplasmic structures like cytoplasmic granules. (All photographs 40X.)

weeks if lymphadenopathy persists. In a small but significant percentage of cases, FNB can produce false negative reports, usually caused by small sample size or, less often, sampling error in cases where the node is partially involved by metastasis or lymphoma. However, most nodes that contain epithelial inclusions or small metastatic deposits or focal lymphomatous involvement are not enlarged or palpable and tend not to show abnormalities on ultrasound, so they are unlikely to undergo FNB.

In cases where the negative or benign FNB diagnosis does not correlate with the clinical expectation or working imaging diagnosis, repeat FNB and or core biopsy, or where suspicion is high for lymphoma or Hodgkin lymphoma, excision biopsy is required. Similarly, an unexpected suspicious or malignant diagnosis on FNB should prompt a review of the clinical and imaging findings, and if the discrepant FNB report cannot be supported by this review or by further ancillary studies or definitive cytological findings then repeat FNB or core should be recommended.

The definition of an adequate smear for the diagnosis of lymphoma is discussed more fully in Chapter 4.

ENDOSCOPIC AND ENDOBRONCHIAL ULTRASOUND-DIRECTED FNB

Since Wiersema reported the first endoscopic ultrasound (EUS)-guided FNB biopsy of a mediastinal lymph node in

1993, the use of FNB to assess lymph nodes and other lesions via a flexible endoscope or more recently endobronchoscope has expanded rapidly to become the standard procedure in assessment of lymph nodes adjacent to the pharynx, esophagus, stomach, duodenum, porta hepatis, celiac plexus or the bronchial tree, and in the mediastinum. Regional lymph nodes draining a primary lesion in lung, pancreas, and gastrointestinal tract, including rectum, can be sampled for an initial diagnosis and staging. For example, EUS and increasingly EBUS can achieve staging of both small cell and non-small cell carcinoma of the lung by assessing posterior mediastinal lymph nodes including subcarinal nodes, as well as the left and, where accessible, the right, adrenal glands. Tissue diagnosis by FNB or core biopsy, where necessary, can be supplemented by appropriate molecular studies for prognostic markers including EGFR and Kras.

The FNB is performed with direct real-time ultrasound that provides assessment of the lymph nodes and has an overall accuracy of around 80% in detecting malignancy. Hypoechogenicity, sharp borders delineating the node, irregular contours of the cortex of the node, increased size, increased short rather than longitudinal axis lengths, and rounded shape with a maximum diameter greater than 10 mm all suggest an abnormal lymph node. Sarcoidosis is suggested by finding clusters of sharply outlined, homogeneously shadowed lymph nodes which are isoechoic with hyperechoic strands.

The advantages of EUS and EBUS FNB are that they are minimally invasive outpatient procedures which are able to predict malignancy in mediastinal and other previously inaccessible lymph nodes in up to 100% of cases, avoiding mediastinoscopy's invasiveness, general anesthesia, higher cost, higher complication rate, and inability to access sites other than the anterior mediastinum, such as the aortopulmonary and inferior mediastinal regions. Where EBUS is available, CT-guided percutaneous biopsies are used only for peripheral lung lesions.

The cost effectiveness of EUS FNB has been documented, and specifically in the staging of lung carcinoma EUS and EBUS FNB can reduce the need for unnecessary thoracotomies for diagnosis or inoperable lung cancers by almost two-thirds. Currently, carcinomas with involvement of contralateral and probably ipsilateral mediastinal lymph nodes are regarded as inoperable. Surgery can be restricted to lung cancer patients with ipsilateral hilar lymph nodes or FNB negative nodes. This diagnostic approach for lymph nodes found on chest X-ray, CT or PET scans has been enhanced by rapid improvements in IHC and molecular techniques applicable to FNB and core needle biopsy specimens to provide definitive tissue diagnoses for both malignant and benign processes.

The EUS and EBUS FNB can diagnose inflammatory or infectious causes of mediastinal lymphadenopathy, including sarcoidosis and mycobacterial and fungal infections, particularly in immune-compromised patients, and EUS FNB can sample spleen, adrenals, and any upper gastrointestinal tumor.

The major disadvantages of EUS and EBUS include the cost of the equipment and training the endoscopist or respiratory physician. Handling the specimen is of paramount importance, as the length of the needle and time in the lymph node can produce significant clotting of the material. Using ROSE not only can establish adequacy and even a provisional diagnosis dictating selection of ancillary testing, but also an experienced cytotechnician or cytopathologist can scrape off clotted casts of the needle from the ROSE Giemsa smears or smears fixed in alcohol for later Pap staining, and place this material along with needle rinses in RPMI or similar for cell block preparation.

The complication rate is less than 0.1%, similar to endoscopy by itself, and includes bacteremia, abscess formation, striking larger vessels, biliary peritonitis, and hemorrhage, which usually occur in elderly patients, and may be related to esophageal strictures and inexperienced endoscopists. Cystic lesions in lymph nodes usually relate to infections, but may be caused by necrosis in lymph node metastases and carry a slightly higher risk of post FNB infections.

Technically, the endoscope has a curvilinear ultrasound transducer at its tip, which provides a longitudinal view parallel to the scope and real-time visualization of the needle as it punctures the lymph node. Doppler assessment of the lymph node also helps to avoid puncturing blood vessels. Once in the lymph node the stylet is withdrawn and the needle should then be moved rapidly backwards and forwards five to ten times, through the lymph node and particularly any abnormality seen in the lymph node, before aspiration is applied, so as to minimize blood contamination of the material. Suction increases the blood contamination and is of doubtful benefit in increasing lymphoid material on the slides.

At smearing, the specimen is split and Giemsa- and Pap-stained smears are prepared. FNB at EUS and EBUS has used 19 to 25 gauge needles but the standard practice is to use a 22 gauge needle, and 19 gauge core needle biopsy