Chapter 8

Fine-needle Aspiration Biopsy

8

Grace C.H. Yang

Contents

Core Features

- Minimally invasive biopsy
- Simple but not trivial
- Accurate diagnosis depends mostly on meticulous technique
- Immediate test turnaround time possible
- Cost effective

Complications to Avoid

- Minor bleeding (intralesional or subcutaneously). This complication can be avoided by advising patients to avoid blood thinners 5 days prior to biopsy; by compression immediately following withdrawal of the needle; or by using 27-gauge needles.
- Infection. This complication can be avoided by using an alcohol wipe and a needle finer than 22 gauge.

Introduction

Fine-needle aspiration (FNA) biopsy is similar to bone marrow aspiration. The samples are aspirated, deposited on microscopic slides, and smeared. Hematologists interpret aspiration smears of bone marrow and peripheral blood, while cytopathologists interpret aspiration smears of the remaining body sites. In addition to the Romanowsky stain used by hematologists, cytopathologist also use Papanicolaou stain for additional nuclear and cytoplasmic details.

The Needle

Conventional wisdom says "the larger the needle, the more abundant the sample obtained." However, it does not apply to FNA, because blood is the biggest enemy of a satisfactory FNA sample. Blood dilutes the cells and makes the sample unsatisfactory (100 cells/0.1 ml of blood is satisfactory, but 100 cells/1 ml of blood is unsatisfactory). Unlike paraffin sections used in histopathology, FNA smears require just a few cells to be diagnostic, but blood aspirated from needles larger than 22 gauge will not only ruin a good sample, but also ruin subsequent samples, because now the blood vessels are ruptured flooding the surrounding area. Optimally, one uses the finest needle possible to obtain a minimally bloody sample which will be diagnostic. Needles smaller than a critical cross-sectional luminal area (Table 8.1) can go in and out of blood vessels as if nothing happened. The elasticity of the blood vessel smooth muscle wall snaps back and seals the puncture holes as soon as the needle passes. One example is acupuncture which can penetrate the human body in various places without causing pain, bleeding, or infection. Acupuncture uses a 27-gauge needle, the same gauge needle most frequently used for thyroid FNA by cytopathologists at the New York University (NYU) Medical Center. The needle is so fine that there is no need to press the puncture site to prevent hematoma. In Fig. 8.1 there is no 22-gauge needle because no 22-gauge needle can be found anywhere in the three FNA clinics at the NYU Medical Center.

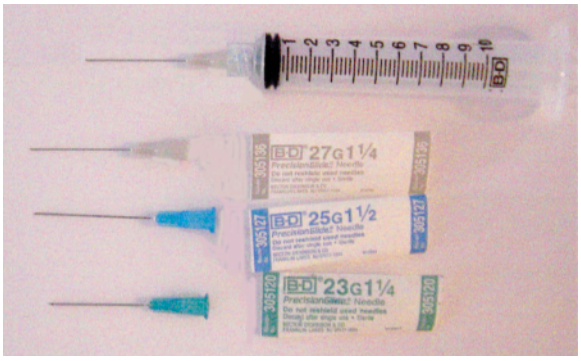

Fig. 8.1: Needles and syringe used for fine-needle aspiration (FNA) biopsy

Fine-needle Sampling Without Syringe

Fine-needle sampling without syringe can obtain cells and tissue particles into the needle hub and is used most frequently by cytopathologists at the NYU Medical Center. This technique was first described in 1987 by Zajdela et al. of Institute Curie in France [17]. This method gives one greatest sensitivity to the changes in the texture of tissues through which the needle passes. The mass is stabilized with the left hand. The fine needle is held by the right thumb and index finger. After the needle is advanced into the nodule, it is moved about in a cone-shaped tissue volume. The needle tip acts like cork borer to cut tiny cores of tissue, which pushed each other into the needle hub. The needle is moved as fast as the needle of a sewing machine. As soon as the sample shows up in the needle hub, the sampling stops. A 10-ml syringe filled with air is attached and the sample is expelled onto microscope slides and smeared. Needle sampling without a syringe is best for small lesions. Although this technique yields less quantity of sample, the quality of sample is better due to minimal dilution with blood. However, the time to obtain an adequate sample is longer than aspiration with suction from a syringe. In addition, it is difficult to sample mesenchymal lesions without suction.

Table 8.1. Diameter of needles used for fine-needle aspiration

Needle gauge	Outside diameter (mm)	Inside diameter (mm)	Cross-sectional lumen area (mm^2)
22	0.71	0.41	0.13
23	0.64	0.33	0.09
25	0.51	0.25	0.05
27	0.41	0.20	0.03

The Syringe

Single use, sterile, disposable plastic 10-ml syringes with a Luer-Lok tip are used in FNA. Larger syringes do not yield larger samples. It is important not to attach the needle too tightly to the syringe, because it slows the removal of the needle and delays smear preparation leading to clotting of the sample.

Syringe Holders

Until late 1990s, the pistol-grip syringe holder, developed in Sweden, was popular. In recent years, the pencil-grip syringe holder [10] (Fig. 8.2) has become popular for clinicians. It is equipped with a release button for automatically drawing back the syringe plunger, and a regulating knob for setting a predetermined amount of negative pressure for the aspiration. It places the hand much closer to the target than the pistol-grip syringe holder, and uses hand movement rather than arm movement. After the needle is advanced into the nodule, a button is pushed and the syringe holder rises automatically to a preset position. Similar to needle sampling without a syringe, the needle tip is moving back and forth cutting fine cores of tissue within the nodule in a cone-shaped imaginary space. The succession of fine-needle cores cut by the needle tip will push each other into the hub of the needle. The vacuum in the syringe facilitates moving of the sample into the needle hub. After the sample appears in the needle hub, the syringe along with the needle is removed from the holder. Surprisingly, the 2- to 3-ml vacuum does not suck the sample from the needle hub into the syringe, because the needle hole has been sealed by the sample. The syringe is then detached from the syringe holder and the needle is separated from the syringe. The plunger is raised all the way up, the syringe is reattached to the needle containing the sample, and the sample is expelled from the needle hub onto the microscopic slides and smears made.

Labeling the Microscopic Slides

Prior to the performance of aspiration, one must label the microscopic slides. Using #2 lead pencil, the frosted end of the slide is labeled with the patient's last name and the number of the nodule. Labeling slides just before aspiration not only eliminates the possibility of confusing patients, but also avoids depositing the sample on the wrong side of the microscopic slide, with the possibility of having the specimen wiped off in the cytology laboratory.

Fig. 8.2: Syringe holder for FNA procedure

The Basic Aspiration Procedure

Fine-needle aspiration biopsy can be adapted to many clinical settings and problems, but the basic procedure remains the same [9]. While the number of passes performed on a given nodule varies, the actual performance of puncture takes less than 10 s per pass in the majority of the cases due to the rapid movement of the needle (as rapid as the needle movement in a sewing machine). The majority of time is spent in locating the lesion, preparing the skin, recovering the sample from the needle hub, and making smears. Despite the procedure's simplicity, individuals new to FNA biopsy should practice the basic procedure using a fruit, such as an orange, until it goes smoothly, quickly, and becomes second nature. The basic aspiration procedure can be broken down into the following steps:

1. Wipe the skin with an alcohol pad.
2. Locate, palpate, and stabilize the nodule.
3. Pass the needle through the skin.
4. Advance the needle into the nodule.
5. Apply suction by raising the plunger, if a syringe holder is used.
6. Move the needle rapidly back and forth, sampling different areas of the nodule.
7. Remove the needle from the patient.
8. Detach the needle from the syringe.
9. Fill the syringe with air.

10. Reattach the needle with the sample onto the syringe.
11. Touch the needle tip to a microscope slide with bevel side down.
12. Express the specimen onto the microscope slide.
13. Make oval smears.

Locate, Palpate, and Stabilize the Target Lesion

Two fingers of the left hand outline and immobilize the mass. In the case of large nodules, only a portion of the nodule is spanned by the fingertips. In the cases of smaller nodules, the entire nodule is encompassed by the fingertips. This approach allows the operator to immobilize the target for the needle to aim. The fingers are arched and extend from the hand, which is poised above a point near the palpable nodule. This makes use of the sensitive fingertips in localizing the target. In this position, the small intrinsic muscles of the hand are relaxed and the fine movements of the fingertips ensure accurate puncture of small targets. Once the nodule is stabilized, the needle is passed through the skin to the lesion. It is helpful to think of the needle as an extension of the index finger of the right hand and the nodule as an extension of the palpating fingers of the left hand. Aiming at a small target is analogous to touching the tip of one index finger with the other. After the needle enters the lesion and suction is applied, the needle is moved back and forth through the mass almost as fast as the needle in the sewing machine. The actual motion causes the needle tip to describe a cone, with its base in the mass and its apex at the entry point of the skin. Always move the needle in a straight line, and change direction at the top of the cone, i.e., near the entry point of the skin. The rapid motion of the needle cuts loose numerous tiny cores of tissue that move up to the hub of the needle. This activity takes only a few seconds. The operator needs to have split vision, monitoring the transparent needle hub as well as watching the reaction of the patients. As soon as material appears in the needle hub, aspiration stops. Depending on the nature of the lesion, in some instances, larger volumes of cyst fluid or blood may be obtained.

Recovery of the Sample from Needle Hub

Hold the air-filled syringe with the left hand and place the needle tip on the glass slide with the bevel side down. Then, using the right hand, carefully push the plunger to deposit a small droplet on each slide, approximately 1 cm from the label. After the sample is deposited onto the slides and smears made, an effort should be made to recover the residual sample adhered to the needle hub by the following maneuver. Holding the needle upward with the left thumb and index finger, use the right thumb and index finger to lift the open end of the needle hub upward and let go, allowing the open end of the needle hub to bounce back onto the microscopic slide so that the adhering material is dislodged onto the glass slide for another smear. One should practice this technique with unused needles filled with creamy hand lotion before applying it to needles containing potentially infectious patient material. The needle can also be gripped with a hemostat, to reduce the danger of an accidental needle-stick injury. The material in the needle hub could be the purest part of the specimen without contamination with blood or fluid. Therefore, recovery, smearing, and study of the needle hub material is important.

Frequently, a small droplet of the sample may be left in the tip of the syringe. It can be recovered in the following manner. The plunger is gently raised all the way up to fill the syringe with air. The plastic tip of the syringe is placed on the glass slide and the plunger is rapidly, forcefully, and audibly pushed all the way down. This maneuver can be rapidly repeated several times so that the droplet at tip of the syringe can be recovered and smeared.

The Fine Art of Smear Preparation

Preparation of smears is the most important steps of FNA. Unless high-quality smears are created, it does not matter how good the samples are, they will be challenging to interpret, because the criteria used for diagnosis by cytopathologists depends on a properly executed smear. First and foremost is to stop aspirating as soon as the sample appears in the needle hub and immediately transfer the sample to the slide and smear, since specimens may dry and a bloody sample may clot.

The smearing technique for FNA is similar to making smears for bone marrow aspirates by hematologists, but more refined. At the NYU Medical Center, the direct smears are made in the Swedish method as taught by the late Torsten Löwhagen of Karolinska Institute in Sweden [9]. The aspirate was deposited 1 cm from the frosted end of the bottom slide. Holding the frosted end with the

left thumb and index finger and the rest of the slide supported by the remaining three fingers, the bottom slide remains horizontal and motionless. Another slide is held by the right thumb and index finger at a right angle to the bottom slide; the top slide is placed on the bottom slide at 45° at a distance so that when the top slide is lowered to 0° in a hinge-like fashion, the majority, but not all, of the droplet will be touched. The untouched area of the droplet serves as an anchor, so that the entire droplet will not roll with the top slide. The top slide then moves over the entire length of the bottom slide toward the preparer in a gentle, quick, and steady fashion. In fact, the top slide glides over the bottom slide along a parallel space filled with air without actually touching the bottom slide. The sample is suspended in a liquid meniscus between the top and bottom slides, so that the cells will be spread apart but not crushed.

Preparation of Multiple Smears from a Single Aspiration by DAB Technique

Sometimes, especially from aspiration of carcinoma or lymphoma, a single pass yields too much sample for one slide and the smear will be too thick for examination. One needs to split the sample into several slides by the DAB technique [11]. These tiny smears will be thin enough for microscopic examination. After depositing a large drop of the sample on the slide, take another slide (the spreader slide) and hold it with right thumb and index finger parallel to the aspirator. The sample deposited on the bottom slide, which is perpendicular to the aspirator, is lightly touched (dabbed) with outer edge of the spreader slide at different spots, starting from the far end of the spreader slide and moving toward the fingers holding the spreader slide. The spreader slide with the newly dabbed droplets can be transferred to three new slides to make three small smears. The last smear is made by flipping over the spreader slide to obtain a new edge, and smear the original slide. In this way, four small high-quality smears can be prepared from one pass.

Although the above descriptions may seem tedious to the reader, they can be mastered with practice and can be executed within a few seconds. Just as the basic motions of the FNA procedure should be mastered before the patient is approached, so should the handling of aspirated material. Smear making can be practiced using droplets of creamy hand lotion. The aspiration can be simulated by material aspirated from an orange.

Gross Examination of the Smears for Specimen Adequacy

Gross examination of the smears is often the ignored step for FNA. Even without a microscope, the operator can gather important information regarding the adequacy of the sample, because the physical features of the aspirates correlate to microscopic findings [16]. Is the sample easy or difficult to expel to the slide? Is the smear easy to make or felt viscous? Typically, the aspirate of pleomorphic adenoma is thick and viscous due to chondromyxoid stroma and Warthin's tumor is thin due to lymphocytes and rare sheets of oncocytic epithelium. Does the smear appear shiny like glazed honey (colloid nodule), thin like water (cystic degeneration), thick like dried tooth paste (epidermal inclusion cyst), or bloody like peripheral blood. Can one observe particles on the smear? If blood appears in the syringe as soon as the needle enters the nodule, make a smear and empty the blood into a test tube containing CytoRich Red and try a different area of the nodule. Never submit slides that the operator already knows by gross examination are unsatisfactory, because cytology laboratories are obligated by regulations to process every single slide that is submitted. Frequently, the cytology laboratory receives a dozen slides, out of which, ten slides appear empty by gross examination. It is the operator's duty to select the satisfactory slides to transport to the cytology laboratory based on gross examination (Fig. 8.3). If the first pass is unsatisfactory, take additional passes until satisfactory, while the patient is still available.

Air Drying the Smears to Create Large, Flat Cells

In the older methods, wet smears were plunged immediately into a coplin jar containing 95% ethanol or sprayed by spray fixative. Air drying was considered bad for cells. In 1988, it was discovered by Chan and Kung [2] that air-dried cells within a certain time period can be restored to transparency by simply soaking in normal saline for 30 s. Ultrafast Papanicolaou stain [14] incorporates this air-drying rehydration by saline technique [13] as the first step, because it allows cytopathologists to examine large, flat, and transparent cells, maximizing the resolution of nuclear and cytoplasmic details and increasing the sensitivity of cancer detection [12]. Figure 8.4 illustrates the cytologic details that can be achieved by this method

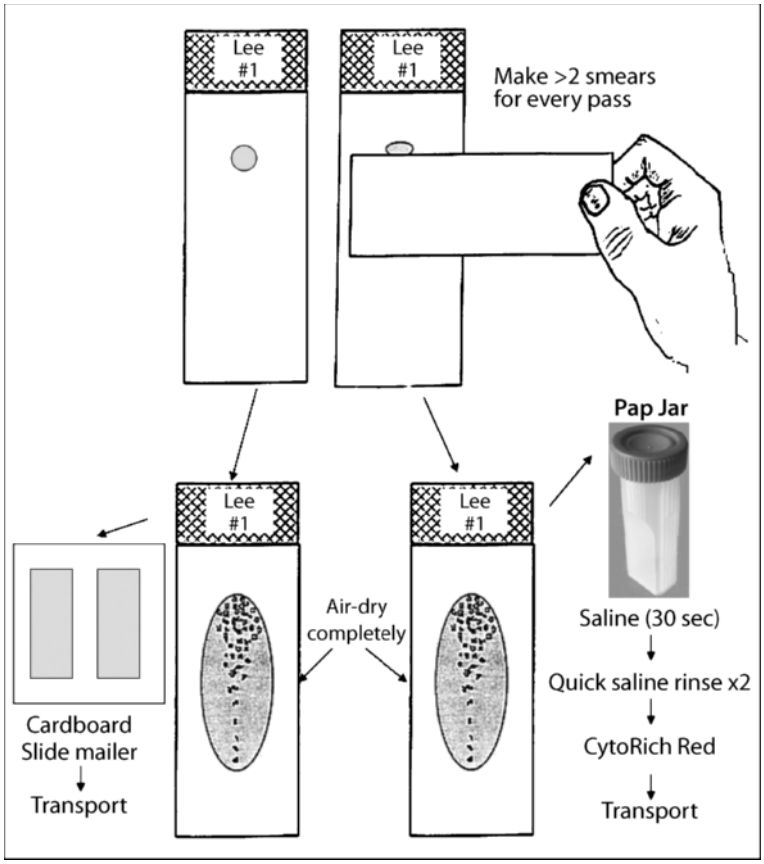

Fig. 8.3: Preparation of FNA smears for transport

and difficult diagnostic problems that can be solved. It is known that approximately 5% of pleomorphic adenomas [3, 4, 7] and some basal cell adenomas [8], share the cylindromatous stroma of adenoid cystic carcinoma on smears. This pitfall is the reason cytopathologists are reluctant to diagnose adenoid cystic carcinoma, and often report "Salivary gland neoplasm with cylindromatous stroma: adenoid cystic carcinoma versus pleomorphic adenoma." This kind of report makes FNA of salivary gland useless to the surgeons, since the former requires radical surgical with wide margins, while the latter uses a conservative approach [1]. The distinction is possible [15] using large, flat, and transparent cells by allowing the smears to air dry followed by rehydration in saline, a simple and elegant technique. In 30 s the saline enters the air-dried cells by osmosis, making nucleated cells transparent and red blood cells hemolyzed. The leaked hemoglobin particles may cover the cells on the smear; therefore, it must be removed by two quick rinses in saline. If left in saline for more than 30 s, the cells will start to detach from the glass surface, so precise timing is important. The cellular details are suboptimal when there is a delay in rehydration by saline. That is why one must rehydrate the smears in saline before transporting the slides in CytoRich Red solution to the cytology laboratory. Figure 8.3 illustrates the steps from the preparation of smears to transport. The air-dried opaque cells transported in cardboard slide holders will be stained in the cytology laboratory for Romanowsky stain to highlight the metachromatic stroma and background substance. The large, flat, and transparent cells transported in Pap jars will be processed for Papanicolaou stain for additional nuclear and cytoplasmic details.

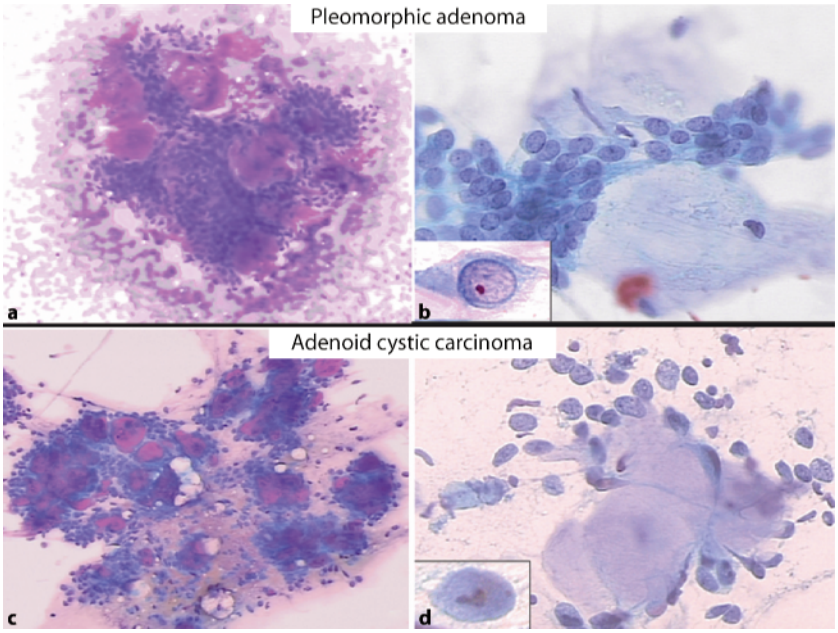

Fig. 8.4: Distinguishing adenoid cystic carcinoma from pleomorphic adenoma in salivary gland aspirates, processed with the method described in Fig. 8.3. **a** Pleomorphic adenoma with metachromatic cylindromatous stroma covered by tumor cells. Romanowsky stain, 100×. **b** The oval neoplastic cells of pleomorphic adenoma have visible cytoplasm. Ultrafast Papanicolaou stain, 400×. Inset Close-up of a tumor cell shows pale nucleus with small round nucleolus and abundant cytoplasm. 1,000×. **c** Adenoid cystic carcinoma with metachromatic cylindromatous stroma. Romanowsky stain, 100×. **d** The cytoplasm of neoplastic cells of adenoid cystic carcinoma measures <1 μm, which is beyond the resolution of the light microscope, thus the neoplastic cells look like naked nuclei. Ultrafast Papanicolaou stain, 400×. *Inset* Close-up of a tumor cell shows no cytoplasm, darker chromatin, and the irregular nucleolus. 1,000×

CytoRich Red is the Cytology Fixative for the Twenty-first Century

All of the previous fixatives, such as 95% ethanol, fix everything in the sample, including nucleated cells, red blood cells, and the background protein. In 1996, an innovative fixative, CytoRich Red, was developed by Maksem [5, 6]. This solution selectively fixes nucleated cells, while paradoxically dissolving red blood cells and background protein. One milliliter of CytoRich Red can lyse 25 μl of whole blood. It is important to know that CytoRich Red will only hemolyze red blood cells when they are wet, and not when they are dried. That is why the air-dried smears must first be immersed in saline before transporting in CytoRich Red, which will dissolve residual hemoglobin particles covering the cells on the smears, providing a clean background for optimal cytologic examination. The rehydrated air-dried cells received in the cytology laboratory can be stained with the standard Papanicolaou stain, and show flat, large, and transparent cells in a clean background for optimal analysis of cellular details.

Liquid Fixation is for Operators Who Cannot Master the Art of Smearing

The sample obtained by aspiration can be directly expressed into liquid fixatives, such as CytoRich Red. Liquid fixation relieves the aspirators from learning to how to make FNA smears, however, the resolution of cellular details is markedly decreased. Using an egg as an analogy (Fig. 8.5), egg yolk is the nucleus of the cell and egg white is the cytoplasm of the cell. The water is boiling in the pot, the eggshell is broken, and the egg is dropped into the boiling water and immediately

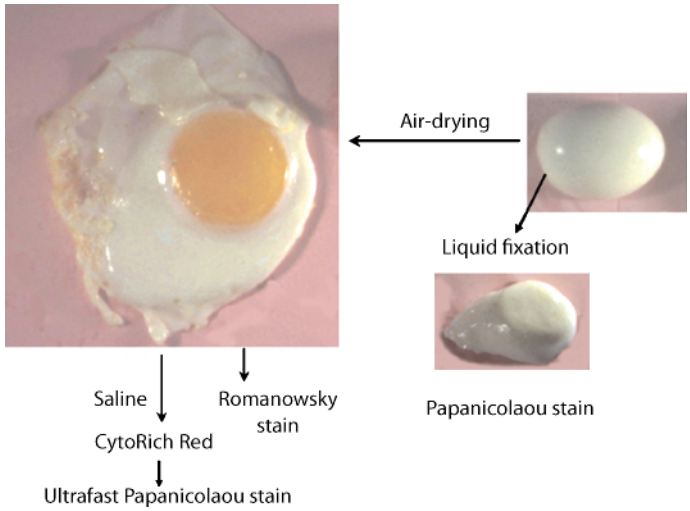

Fig. 8.5: Cytology preparation is analogous to preparation of eggs. Highest resolution of cytologic details is possible only with air drying, which provides the largest cytoplasm and nucleus for cytomorphologic analysis. Cells placed in liquid fixation are like poached eggs with smaller nuclei and cytoplasm, making it difficult to distinguish look-alike entities

cooked into poached egg. Liquid-fixed cells are analogous to "poached egg" with smaller nuclei and smaller cytoplasm under the microscope. Air-dried cells are like "sunny-side up egg." The eggshell is cracked and the egg is dropped over a flat pan. The egg flattens by gravity and is then cooked into sunny-side up egg. Air-drying allows the cells to flatten by gravity. The flattened cells appear larger under the microscope for higher resolution of cytoplasmic and nuclear details. In addition, liquid fixation precludes the use of Romanowsky stain, the most popular stain for cytopathologists, and the use of Ultrafast Papanicolaou stain, the stain that provides the highest resolution of nuclear and cytoplasmic details. Placing fine-needle aspirates in liquid fixation for cytopathologists is like placing bone marrow aspirates into liquid fixative for hematologists. The exquisite cellular details shown in Fig. 8.4 for optimal cytomorphologic analysis and to distinguish look-alike entities will not be possible in liquid fixation.

Needle-rinse Material Contributes Little to FNA Diagnoses

Although routine preparation and examination of slides from needle-rinse material may be unnecessary and inefficient, collecting this material at the bedside requires very little additional time or effort. Thus, some operators routinely rinse the needle and syringe at the time of FNA and then process this rinse-material specimen only in rare cases in which additional material is needed.

Role of FNA Biopsy to Diagnose Lymphoma of the Parotid Gland

With the advent of sophisticated cellular immunological methods, such as flow cytometry, characterization of different cellular subsets within the aspirate population can be achieved by staining for surface markers using fluorescent antibodies. Collection of a least two needle passes in Hanks' basic salt solution or Roswell Park Memorial Institute (RPMI) medium for immunophenotyping by flow cytometry addresses some of the concerns with adequacy of FNA biopsy specimens. Immunophenotyping by flow cytometry detects cell surface antigens using a variety of monoclonal antibodies that indicate differentiation, lineage (B or T ells), and whether there is a monoclonal production of the light chains or the heavy chains. Immunophenotyping also confirms the reactive nature of the lymph node because there will be a polyclonal pattern of immunoglobulins with production of both light chains with a kappa:lambda ratio of 2:1. Gene rearrangement studies can be performed when immunophenotyping is equivocal, and this has resulted in a substantial increase in accuracy for typing lymphomas. These are the genes for cell surface antigen receptors, and arrangement of these genes is the earliest genetic event in the development of lymphomas. The gene rearrangement can be detected using the Southern blot technique or polymerase chain reaction. Even if an open biopsy of a lymph node has to be performed following a FNA biopsy, reactive, inflammatory, and metastatic lesions have been eliminated and the diagnostic possibilities are narrowed down.

Take Home Messages

- BLOOD IS THE ENEMY: use the finest needle possible to avoid dilution of tumor cells by a tsunami of blood.
- LESS IS MORE: a scanty undiluted sample is better than an abundant blood diluted sample.
- THE CLOCK IS TICKING: work fast to avoid clotting of the sample before smearing.
- GROSS INSPECTION of smears for adequacy and correlation with final diagnosis.
- METICULOUSLY FOLLOW DIRECTIONS which will ensure success of FNA.

Supplies for FNA Biopsy

Alcohol pads:
- Sterile, alcohol prep pad, reorder #:122
- PSS Select, Jacksonville, FL 32216

Needles:
BD PrecisionGlide needles, single use
- Cat# 302136 27 gauge, 1-1/4"
- Cat# 302127 25 gauge, 1-1/2"
- Cat# 302120 23 gauge, 1-1/4"

Syringes:
BD 10-ml plastic disposable syringe with Luer-Lok tip
- Ref 309604
- Becton-Dickinson, Franklin Lakes, NJ 07417
- Becton-Dickinson, http://www.bd.com

Pencil-grip syringe holder:
- Tao aspirator, Tao & Tao Technology, 886 Sands Lane, Camano Island, WA 98282; Tel.: +1-360-3876186, http://www.taoaspirator.com

Pistol-grip syringe holder:
- Cameco Syringe holder, Belpro Medical, 9915 Place York, Anjou, Quebec, Canada Tel.: +1-888-2301010, http://www.belpro.ca/com

Microscopic slides:
- Newcomer Supply, 2505 Parview Road, Middleton, WI 53562; Tel.: +1-800-3837799
- Cat# 6215 Microscopic slides with frosted ends, 3×1×0.1 cm

Saline:
- Baxter Healthcare., One Baxter Parkway, Deerfield, IL 60015; Tel.: +1-800-4229837, http://www.baxter.com/
- Cat# 2F7124 0.9% NaCl, irrigation saline, USP

CytoRich Red preservative:
- TriPath Imaging, 780 Plantation Drive, Burlington, NC 27215; Tel.: +1-866-8747284, http://www.tripathimaging.com/
- Cat# CytoRich Red preservative 3600 ml/bottle

Pap jars:
- Evergreen Scientific, 2300 East 49th St., PO Box 58248, Los Angeles, CA 90058; Tel.: +1-800-4216261, http://www.evergreensci.com/ch/pfp.htm
- Cat# 240-5420-G8K Pap jars w/ green caps, empty, pack of 200

Cardboard slide mailers:
- Richard-Allan Scientific, Kalamazoo, MI 49008; Tel.: +1-800-5227270, http://www.rallansci.com/
- Cat#2500 Slide Mailer

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