Ultrasound-guided fine needle aspiration biopsy: does cytopreparatory technique influence specimen adequacy?

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Purpose

1- Fine needle aspiration (FNA) biopsy is a technically simple outpatient clinical procedure, which usually does not even warrant the use of local anesthesia. Samples, usually of rich cellularity, can be obtained from superficial or deep targets, with or without the use of imaging guidance, and used for both morphologic assessment and a variety of ancillary tests.

2- Although the FNA procedure is relatively simple, its component parts are not trivial, and the overall efficacy of the technique depends on:

a) the ability of the operator to obtain a representative and cellular sample from the lesion,

b) the quality of the cytopreparatory techniques used,

c) the familiarity of the pathologist with cytomorphologic features of the lesion at the target site.

3- Guidelines and standards exist for the performance of FNA biopsies, especially in commonly sampled sites. For example, training for the performance of thyroid FNA under ultrasound imaging recently discussed among multidisciplinary committees of the National Cancer Institute [ref 6]. Sample preparation techniques however are not standardized and some interventional radiologists prefer to prepare direct smears on glass slides (classic smears, CS) and others to place the entire cellular harvest into an alcoholic fixative such as those used for cervical cytopathology (liquid based preparation, LBP).

4- Very few is known about the adequacy rate of using different cytopreparatory techniques in a clinical set. Therefore, the purpose of this study was to evaluate the specimen adequacy rate obtained in a cohort of consecutive ultrasound guided FNA biopsies (US-FNA) performed by a single radiologist in two settings: a tertiary hospital with cytopathologists experienced in using CS and a separate tertiary hospital with cytopathologists experienced in the interpretation of LBP.
Methods and Materials

This is a retrospective review of FNA database prospectively collected and selecting the cases performed by the same operator when using sonographic guidance. The study population was made up 2,800 consecutive FNA biopsies, where 375 US-guided FNA in the institution A and 279 US-guided FNA in the institution B. The average age of the patients was 41 years ± 12.4 (SD). 68% were female and 32% were male patients.

**US equipment(s):** Any commercial available sonographic equipments with a linear transducer 5-10 MHz. Gel was used during the scanning but sterile gel not used during the interventional procedure in either institution.

Sterile gel was not used as it can clog the aspiration fine needle and interfere in the processing of the cytological material (Figure 1 on page 5). Any sterile liquid was used for the contact with the transducer after asepsis, including the antiseptic liquid itself used. Most of the times a sterile alcohol-based swabs were used. In all procedures a rubber condom without lubricant was used to cover the transducer and latex gloves were used.

**Biopsy procedure:**

In both institutions the lesion was identified by palpation or by sonographic assessment. Then, a 22-gauge needle for breast lesions, and 25-gauge needle for other superficial organs or structures, was advanced into the target.

For all biopsies a manual cyto-aspirator 10-ml syringe pistol fitted with the 10-ml BD syringe (Cameco, London, UK) was used to perform a vacuum assisted biopsy. After confirming the tip of the needle was inside the targeted area, maximum vacuum was applied and the lesion was considered adequately sampled when some material seen in the hub of the needle. The suction was released and the entire device removed (Figure 2 on page 5).

**Cytopreparatory technique:**

In the hospital A, the samples obtained were expelled onto a glass slide (Figure 3 on page 6, Figure 4 on page 7), smeared into a thin layer (on page 8, Figure 5 on page 7, Figure 6 on page 9), and either fixed with 95% ethyl-alcohol (Figure 7 on page 10) and stained with modified Papanicolaou stain or air-dried (Figure 8 on page 11) to be used for Romanowsky stains. The operator, a board certified and fellowship-trained radiologist with 20 years experience doing imaging-
guided interventional procedures, made an average of 3 glass-smears/lesion (min 2 and max 5 smears). Visual inspection of the FNA classic smear technique can be done by the radiologist if it is done by an assistant (Figure 9 on page 12). An assistant was not used in this study and the smears prepared by the radiologist.

In the hospital B, the samples collected were not immediately prepared onto glass slides on site, but instead were flushed into CytoLyt® fixative by the operator and sent to the lab.

When a morphologic study with conventional stains was not sufficient for a definitive diagnosis, the same cytologic samples (Figures 10 and 11) prepared by either technique could be used for immunohistochemical cell typing (Figures 12 and 13) at both institutions.

**Diagnostic Criteria:**

In the hospital A, specimens were considered non-diagnostic if insufficient cellular material—that is, fewer than six groups of cells containing > 10 cells each—was present and no evidence of cellular atypia was found. The pathology review was done by a pathologist with 22 years of experience and sub-specialized training in cytopathology with Torsten Löwhagen, Sweden school.

In the hospital B, FNA samples were considered non-diagnostic if the staff cytopathologist rated them as insufficient based on review of the slides. Pathology review in institution B was done by three pathologists with 20 to 26 years of experience in cytopathology, two of them with sub-specialized training in cytopathology with Torsten Löwhagen, Sweden school.

The figure 14 on page 17 show an example of non-diagnostic exam. With Thin-Prep slides the individual cells may be quite dispersed and must be evaluated on an individual basis.
Fig. 0: The use of gel during the ultrasound-guided FNA is not advised as the gel is suitable to be stained and may mimic areas of necrosis when reading the slides on the microscope. The 2 glass slides presented in the upper right corner are very similar using naked eyes, however one is from a thyroid nodule aspirated and the other was made from sterile ultrasound gel exclusively. Ultrasound gel could stain like a colloid goiter aspirated material.
**Biopsy procedure**

Manual cytoaspirator 10-ml syringe pistol fitted with the 10-ml BD syringe

Multiple insertion of the needle on the same direction

**Needles:** 22G (breast), 25G (other superficial organs)

**Fig. 0:** FNA Biopsy procedure sequence. This is a vacuum assisted biopsy (VAB) using a much thinner needle (22-25 G) in order to obtain a cohort of cells, not tissue sampling.

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**Fig. 0:** Smear preparation 1.

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Fig. 0: Smear preparation 2.

Place cellular material in one “dot” near the frosted end of the slide.

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Fig. 0: Smear preparation 3.

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Fig. 0: Smear preparation 4. Hold the slide with the specimen on it at the corner. In preparation of one smear one edge of the upper smearing slide is placed on the lower slide and it is then gently lowered onto the dot of material. (Think of the lower edge of the smearing slide, nearer your hand, as being on a hinge. Minimal (if any pressure) should be exerted. The cellular material should spread by capillary action. After the material has started to spread draw the upper smearing slide down the length of the lower slide, keeping it absolutely parallel. The result should an oval shaped monolayer of material on the lower slide - and virtually nothing on the upper slide.

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Fixation of a wet smear for H&E or Pap stain is better achieved by dropping the slide into a Coplin jar of alcohol.

**Fig. 0:** Smear preparation 5. Fixation in wet material needs to be immediate, as soon as you finish smearing a thin layer of the aspirated material, drop the glass slide in the preservative available (Saccomano, Cytolite, alcohol, etc).

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Fig. 0: Smear preparation 6. Air-dried fixation. Slides can be placed in an empty jar after stayed for few minutes over the counter when radiologist is placing the order, typing the online request or filling manually the paperwork. However, in order to speed up a Cytospray (women's hairspray) can be used when safety conditions available.

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Fig. 0: Visual inspection of the smears, in this case after processing by the lab. The "ideal" smear (a) should have smooth margins and an oval shape monolayer of the aspirated specimen. See also sequence of smear preparation in this ePOS. The glass slide presented in (b) has irregular shape and several layers with non uniform thickness. It is suspected that too much aspirated material (instead of a single dot) was placed and smeared with minimal pressure in starting smearing and too much pressure was obtained at the end of the preparation.
Fig. 0: Highly suspicious 6 x 4 mm hypoechoic non-palpable mass in the breast. US-FNA performed using 22G needle and classic smear technique preparation.

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Fig. 0: Images that we radiologists do not see frequently. What happened after classic smear cytopreparatory technique? Digital photography of the glass slide after Papanicolaou stain was used, 5x magnification showing several papillary arrangements, 10 x magnification detail of papillary feature, 40 x magnification with nice nuclear morphology and preserved background. Please note that LBP tends to wash the background and morphology needs to be rearranged using cell blocks.

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Fig. 0: Immunohistochemical cell typing of the case presented in Figures 10 and 11.

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**Fig. 0:** Immunohistochemical cell typing of the case presented in Figures 10 and 11.

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**Fig. 0:** Non-diagnostic material. Evaluation limited by low cellularity. The individual cells collected using liquid based preparation are quite dispersed and must be evaluated on an individual basis. Note that the background is not present as seen in the case presented in Figures 10-13.

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Results

654 FNAs (23%) were preformed under ultrasound guidance and 2,146 (77%) were performed without imaging guidance (palpable lesions).

The site location of US-FNA was 41% breast, 30% thyroid gland, 22% lymph node (any place in the body), 5% salivar gland, 2% soft tissue. All lesions aspirated were masses, with or without associated calcifications, with homogeneous or heterogeneous (complex solid cystic masses) internal echoes. Aspirated material of symptomatic breast simple cysts were discharged and not included in this database.

Lesion size ranged from 3 to 19 mm, mean 8.36 mm. US-FNA using LBP exclusively mean size was 7.87 mm and US-FNA using CS was 8.72 mm.

With exclusive use of LBP, 221 of 279 (79%) specimens obtained under sonographic guidance were considered adequate for cytomorphologic evaluation. With use of CS technique, 369 of 375 (98%) specimens obtained under sonographic guidance (Figure 2 on page 20). If considered the overall adequacy rate using classic cytopreparatory technique 95% of 2,517 FNA specimens were considered adequate.

The difference in inadequacy rates of specimens assessed by LBP (20.78%, 95% CI,17.42%-24.30%) versus those assessed by conventional smears (1.6%, 95% CI, 0.9% - 2.91%) in ultrasound-guided FNA biopsies was found to be statistically significant (p< 0.001).
Fig. 0: Distribution of US-FNA by organ aspirated and percentage of adequate / inadequate sampling by cytopreparatory technique used. Please note that LBP was used only in Breast and Axillary Lymph Nodes.

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Fig. 0: Distribution of US-FNA adequacy rate based on cytopreparatory technique used (CS, classic smears versus LBP, liquid based preparation).

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Conclusion

LIMITATIONS:

• Areas sampled are diverse (53% of A were thyroid biopsies and 70% of B were breast biopsies).

• We acknowledge that high or low adequacy rate cannot guarantee accuracy without knowledge of the outcomes (surgical removal, core biopsy or long term follow-up).

• A prospective study involving sampling of the same area with preparation of smears and LBP would be ideal, but would be difficult to justify ethically. This study did achieve statistical power.

CONCLUSIONS:

• In 2 series of Vacuum-Assisted US-FNA performed by the same operator there was a higher inadequacy rate when cytopreparatory LBP technique was used.
• The use of direct smears for US-FNA may impact the clinical utility of this interventional procedure.
• Further studies addressing optimization and pre-analytic standardization of US-FNA are necessary.
References


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