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Editorial

FNC Thyroid: The Pitfalls of Fine Needle Cytology Sampling. Procedure to Obtain Higher Diagnostic Accuracy by Fine Needle Cytology of the Thyroid

Di Benedetto G*
Department of Clinical Pathology, Second University of Naples, Italy

***Corresponding author:** Di Benedetto G, Department of Clinical Pathology, Cytopathology Service, Clinical Hospital of Second University of Naples, Italy, Tel: +39-081-500-1355; Fax: +39-081-500-1329; Email: diben.g@libero.it

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Contrary to the belief that is held by many clinicians that aspirating the thyroid is an easy and simple task, the performers of FNC often commit several cardinal mistakes. Several studies [1-3] have investigated the effects of the sonographic features and needle size to obtain adequate cytological material by fine-needle aspiration cytology of the thyroid nodules. Recently a study [4] concluded that nodule size and needle size used for sampling did not affect the adequacy of FNC.

These studies, although interesting, do not take consider the opinion of the cytologist with regard to the preparation of the sample, which is a crucial for the execution of a cytologically correct diagnosis [5,6]. As a result, FNC cytology accuracy of the thyroid is impaired. The operators often commit several cardinal mistakes that have a negative influence on cytologic diagnosis:

- The aspirates take too long and samples are diluted with blood
- The use of larger calibre needles improves blinding that obscures the cell sample.
- Inadequate Samples for a cellular sample of epithelial cells.

Therefore, I think that it is necessary to do a briefing to focalize some fundamental issues on FNC sampling of Thyroid.

Needle Caliber

Aspirations of the thyroid are best performed with 25 or 23 gauge needles by two experienced operators; a cytopathologist and ecographist. The use of larger calibre needles is wrong because they cause more bleeding and result in unreadable bloody aspirates [5,6]. Also, the smallest calibre needles preserve the cytological architecture of the thyroid nodule and for the cytologist this is of paramount importance for the diagnosis of follicular lesion because the follicles and papillary features are more easily recognizable using the smallest calibre needles.

Fine Needle Cytology

After insertion, the needle is rapidly moved in and out without suction. Because the thyroid is richly vascularized, the needle cytology must be performed as rapidly as possible, in about 2 seconds in order to avoid dilution of samples with blood [6].

The preparations have be smeared by a pathologist onto one glass slide, air dried and stained with Diff-Quick [6].

Preparation of Smears

I recommend following the Swedish school of cytology and favour air dried smears fixed in methanol and stained with hematologic stains. The preparations have be smeared by a pathologist onto one glass slide, air dried and stained with Diff-Quick and microscopically evaluated immediately while the patient waits and , only if the aspirate is inadequate for accurate diagnosis , should the patient be re-aspirated. The needle should be washed by aspirating 2 ml of physiologic solution which is then collected into a tube [7]. The material can then be used for molecular testing [7].

The advantage of this technique is:

1. The short preparation and staining time and excellent quality of nuclear features.
2. The sample is adequate to facilitate a correct cytologic diagnosis
3. It is possible to perform V600- Braf Test on the material collected for molecular test on indeterminate sample [7].
4. The inadequate sample disappears.

Molecular Biology

On indeterminate cytology diagnosis, the cellular material collected for molecular testing can be selected by centrifugation and used for DNA extraction.

Extraction

DNA can be obtained with a salting-out method [7]. Purity can be assessed by spectrophotometry (Biophotomaker, Eppendorf), while the degree of integrity can be evaluated with electrophoresis using 100 ng of DNA extract [7].

V600-BRAF mutation

A mutation study has performed by amplification refractory mutation system PCR. One hundred nanograms of DNA was subjected to 35 cycles of amplification using a reaction mix containing, in a final volume of 50 µl, 10 mmol of Tris-HCl, pH 8.3, 50 mmol of KCl, 200 µmol of deoxynucleotide triphosphates, 1.5 mmol of MgCl 2 , 25 pmol of each primer and 1.5 IU of Taq Polymerase (Euroclone 5 UI/µl).

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Confirmation of the V600-BRAF mutation was determined with gene sequencing (Biosystem kit) and scanning on an automatic analyzer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems). For the evaluation of the validity of DNA samples, we then amplified a region of the thyroglobulin gene.

Discussion

The FNC collection protocol described here has been proved to be highly efficient [7]. Cytological and molecular tests gave adequate results in 100% of cases, considering that adequacy has been evaluated for each patient by a pathologist. In particular, by using a 23-gauge needle and moving it up and down several times we get a sufficient and high quality amount of material that is partially smeared and stained on a slide and partially suspended in a solution of 0.9% NaCl. So, without affecting the diagnosis, we extracted useful material for molecular biology and for V600-BRAF mutation analysis. With regard to the cytological diagnosis, the slide set up after fine needle insertion and stained with Diff-Quick shows enough material for a correct diagnosis [7]. In addition, we performed testing for the V600E-BRAF mutation on all samples using the left over material from the needle; the amount of DNA extracted was sufficient to research the mutation.

We believe it is important to extract DNA from the same specimen as it will surely be representative of the same lesion. With a one-time withdrawal thyroid FNC, we obtained cytological and molecular data that may be useful for both the diagnosis and prognosis of the patient.

Conclusion

In this editorial, I want to stress that it is needful, for the good performance of the thyroid FNAB, the simultaneous presence of the sonographer and the cytopathologist expert, in full according with the Swedish School of Cytology [5,6].

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