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 nmol of Tris-HCl, pH 8.3, 50 nmol of KCl, 200 μmol of deoxynucleotide triphosphates, 1.5 mmol of MgCl2, 25 pmol of each primer and 1.5 IU of Taq Polymerase (Euroclone 5 U/μl).
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].

References

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