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The sequential application of immunocytochemistry, BRAF-1 and N-RAS mutation analysis identifies malignant follicular thyroid neoplasm's on liquid-based cytology

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Objectives: Fine needle aspiration cytology (FNAC) is an important tool for evaluating thyroid nodules although up to 20% of cases may result in an indeterminate diagnosis (Follicular Neoplasm - FN) which is a challenge also for expert cytopathologists. The possible role of immunocytochemistry (ICC) in dividing "low and high risk" FN has been largely discussed. Furthermore, some molecular alterations of specific pathways as playing a pivotal role in thyroid cancer onset. *BRAF, RET/PTC or N-Ras mutations* are referred as markers of malignancy for papillary carcinoma (PTC), the most common thyroid malignancy. *BRAF* mutations have been identified in 70% of classic and tall cell variants of PTC but only in 20% of its follicular variant (FVPC). The point mutation in Ras genes (H-Ras, K-Ras, and N-Ras) has been highlighted in thyroid neoplasia with an overall different incidence according to the histotype. We aim at the diagnostic role of ICC including HBME-1 and Galectin-3 as a first step in a sub-classification of FNs in order to detect *BRAF* and *N-RAS codon* 61 mutations analysis in the "high risk"FNs.

Methods: From October 2011 through September 2012, 30 cases diagnosed as FN (12 Benign and 18 malignant at histology) processed with liquid based cytology (LBC, Thin Prep, Hologic, USA) underwent surgery at the Catholic University of Rome (Italy). In all cases ICC for HBME-1 and Galectin-3 was carried out and followed by *BRAF* and *N-RAS codon 61* mutational analysis in those resulted BRAF wild type. ICC as well as DNA extraction were performed on LBC-stored material.

Results: Our series express ICC positive complete panel in 24 FN cases (all 18 malignant and 6 out of 12 benign), while the 6 remaining benign showed only HBME-1 positivity. Two cases expressed *BRAF* mutation while the *2 RAS* mutated cases were diagnosed as Follicular variant of PTC (FVPTC).

Conclusions: The sequential application of ICC and mutational analysis on LBC may enhance the accuracy of FNA and its cost-effectiveness. ICC may be use as a discriminative limit of "low and high risk" FN lesions. For this category the use of molecular testing can be apply on high risk lesions providing more malignancies, especially when supported by RAS analysis for identifying the FVPTCs. The cases negative for ICC can benefit of a strict follow-up instead of surgery. This mutational panel might help in guiding a more aggressive surgical approach.

Biography

Rossi has completed her M.D. at the age of 25 years. She is the winner of many national prizes and awards. She studied for some different periods in Philadelphia, Paris and Porto focusing in her studies on application of immunotechniques and molecular analyses. She has published more than 35 papers in reputed journals and serving as an editorial board and reviewer member of many repute. She has been invited international speaker in more than 20 international meetings. She is authors of some chapters books and author and editor of the Cytopathology for the Encyclopedia of Pathology-Springer.

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