

Multiplex PCR approach to simultaneously identify several mutations in fine needle cytology thyroid samples

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ABSTRACT

The most frequent initial manifestation of thyroid cancer is the appearance of a nodule. More than 20% of the general population has a palpable thyroid nodule and the percentage rises to 70% based on ultrasound identification. In 95% of cases the nodule is simply a hyperplastic or benign lesion. The most reliable diagnostic test for thyroid nodules is fine needle aspiration (FNA), but cytological discrimination between malignant and benign follicular neoplasms remains difficult. Cytological analysis is now, almost routinely, being combined with molecular genetics to enable the pathologist to make a more objective diagnosis. In this study, we performed the molecular analysis using a new simplified procedure that involves a panel of *BRAF*, *RAS*, *RET* and *RET/PTC* gene mutations in easily obtainable FNA samples, in the attempt to improve the efficacy of the FNA diagnosis of thyroid nodules and thus patient management. In this new procedure, PCR and sequencing analysis are used to detect point mutations, and, in parallel, RT-PCR is used to detect the chimeric *RET/PTC1* and *RET/PTC3* transcripts in RNA extracted from FNA.

INTRODUCTION

Thyroid cancer is the most common malignant tumor of the endocrine system [1]. Most thyroid cancers derive from thyroid follicular cells that give rise to well-differentiated papillary (PTC) and follicular (FTC) carcinomas, and undifferentiated forms such as anaplastic carcinoma (ATC) [2], while medullary thyroid carcinomas (MTC) derive from thyroid parafollicular cells or C cells. Medullary thyroid carcinoma accounts for approximately 5–10% of all thyroid cancers, 75% are sporadic and 25% hereditary. A nodule is usually the initial manifestation of thyroid cancers but in 95% of cases it is simply a hyperplastic or benign lesion. Therefore, given the low

mortality rate of thyroid cancer and the low percentage of malignant thyroid nodules, an accurate diagnosis of lesions will spare patients unnecessary surgery.

Although thyroid nodules are mostly diagnosed by fine needle aspiration (FNA), the cytological discrimination between malignant and benign follicular neoplasms remains difficult despite their distinctive cytomorphological features, PTC, MTC and ATC are easily diagnosed. However, FNA may yield insufficient material or a low number of tumor cells, and many thyroid FNAs are "indeterminate". In the latter case, FNA sampling is usually repeated or in some cases, surgery is performed in the attempt to establish a diagnosis. These procedures result in additional morbidity and higher health