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BRAF Mutation in Papillary Thyroid Cancer

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Abstract

Purpose of Work: As a highly specific mutation of papillary thyroid cancer (PTC), BRAFV600E dosage in thyroid fine needle aspiration cytology (FNAC),has been shown to be useful in indeterminate cytology.PTC represents the most common histotype of thyroid cancers (85-90%). In these tumors, the most frequent genetic alterations are those responsible for the oncogenic activation of genes such as B-RAF, RET, NTRK1, RAS, MET, and silencing of oncosuppressive genes such as p53, RASSF1-A, PTEN, PPARY and CDK inhibitor. B-RAFV600E is present in approximately 50% of PTCs, responding with more aggressive variants of this cancer in terms of invasion, clinical stage, risk of recovery and sensitivity to radio iodine therapy.

Materials and Methods: We conducted a prospective randomized study on 50 patients who attend to our structure from 2013 to 2016 in order to study the predictive effects of the B-RAF mutation on fine needle aspiration cytology (FNAC) in patients with nodular thyroid disease.

Results: From our study we can see that the molecular dosage of B-RAF is superfluous when the cytomorphological examination is based on characteristics of benignity (Tir 2) or malignancy (Tir 5); it is of great help in cases of indefinite (Tir 3) or suspicion nodule (Tir 4), even though its negativity does not allow us to exclude a cancer, its positivity gives us a diagnosis of certainty.

Conclusions: Improvement of FNAC's diagnostic accuracy through the molecular dosage of mutated B-RAF represents a valuable achievement for the management of patients with nodular thyroid disease with clinical-instrumental features of suspicion both for preoperative and postoperative strategies, permitting us to quickly dispel the uncertainties of the cytomorphological examination and allowing to start the patient towards the most appropriate therapeutic strategy.

Introduction

New techniques of genetic engineering have allowed to identify alterations to genes involved in tumor pathogenesis, which, in addition to affecting the course of the disease, also characterize the more or less aggressive phenotype. In the field of thyroid disease, the most common ones are those responsible for the oncogenic activation of genes such as B-RAF, RET, NTRK1, RAS, MET and silencing of oncosuppressive genes such as p53, RASSF1A, PTEN, PPARy, and CDK inhibitors. RET; NTRK1, B-RAF and RAS are involved in the cascade of MAPK (Mitogen Activated Protein Kinase) signals. [1-4] The oncogenic mutation of B-RAF is the predominant genetic event of thyroid papillary carcinoma, present in 44% of cases with a variability of 28-83 % [5,6]. Several studies indicate that B-RAF mutations are associated with increased tumor aggression in terms of invasion, clinical stage and relapse risk [5-12]. Various authors found a correlation between the T1799A mutation in B-RAF and the mostsevere

clinical pathological characteristics of the disease and this leads us to evaluate this alteration as a genetic marker of malignancy, useful both in diagnosis and therapy orientation. [9,10,13,14] A Chinese epidemiological study has shown that in high-injected areas of iodine there is a significantly higher incidence of BRAF V600E mutation (69%) than normal intake areas (53 %). These data suggest that iodine intake is a risk factor for the BRAF V600E mutation and can therefore be a risk factor for the development of papillary thyroid carcinoma PTC [13]. Numerous studies on the relationship between BRAF V600E and the clinical-pathological characteristics of PTC are available in the literature. Most of them have suggested that the BRAF V600E mutation is associated with advanced disease stages (III-IV) and aggressive phenotype while others have not found this correlation. The association with the extracellular extension and the development of lymph node metastases have also been found, resulting most commonly associated with the BRAF V600E mutation [1,12,14-18].

A 2005 study found that BRAF mutating itself would not be enough to induce an aggressive biological behavior of the tumor: the mutation would induce some genomic instability and a greater predisposition of mutated cells to acquire further defects (e.g., RAS mutations) together, it can explain the increased aggressiveness found in B-RAF mutated PTCs [16]. Finally, some studies, including a multicenter study of 219 patients, demonstrated that the B-RAFV600E mutation is an independent predictor of tumor recurrence after a variable follow-up period between different studies [1,12,16-18].

The relief of the B-RAFV600E mutation could also be useful in the diagnosis of PTC on DNA samples obtained from cytological withdrawal with FNA, as confirmed by a study by Salvatore, et al. [19] where mutation identification has been found to have enabled PTC diagnosis in 5 out of 15 samples previously considered undetermined or insufficient for cytological diagnosis. Two other studies by Marchetti, et al. [20] and Zatelli, et al. [21] have shown that the combination of traditional cytology with the molecular analysis of the B-RAFV600E mutation on FNA samples has improved PTC's diagnostic accuracy from 62.3 % to 82.2% and from 77.3% to 86.7% [20,21]. However, the B-RAFV600E mutation is only positive in about 50% of PTC, and in the case of a negative result one cannot rule out a malignanttumor.

It is understood therefore that preoperative research of this mutation, alongside cytomorphology, may be of utmost utility in the diagnostic-therapeutic approach of PTC. The 2015 American Thyroid Association (ATA) guidelines simply provide a recommendation for the use of B-RAF as a molecular indicator in an attempt to improve clinical management in patients with FNAC displaying undetermined cytology [22]. The use of B-RAF may also be helpful in guiding therapy, as it is useful not only in selecting patients with thyroid disease to undergo surgery, but also in modulating PTC treatment, which uses surgery and ablative metabolic radio-iodine therapies [23-24]. Indeed, several studies have shown that the B-RAFV600E mutation is associated with a high risk of recurrence and a reduction in iodine [8,16,23-26].

Materials and Methods

We selected 50 patients with nodular thyroid disease who attended our structure from 2013 to 2016. Patients were divided into two homogeneous groups of twenty-five each. In both groups, the cytological examination of the aspiration was performed on microscope slides assembled and fastened to air. Non-diagnostic results were repeated. In the patients assigned to the experimental group, the BRAFV600E molecular assay was further performed. As a criterion for inclusion in the study, the presence of at least one ultrasound suspect characteristic of the solid nodule examined was considered, ipo echogenicity, irregular and faded margins, presence of intra-lesional microalcifications, vascular pattern III according to Lagalla. The only criterion considered for exclusion was the anechogenicityof the nodule: that is, the cystic colloidal nodules were excluded from the study, provided there is no protruding solid vegetation in the lesion. All the withdrawals were performed by the same operator and the preparation and reading of the slides were all made by the same cytopathologist.

Results

In the experimental group, from the cytomorphological point of view, 14 nodules (58%) appeared benign (tir2), 4 (16%) undetermined (tir 3), 3 (10%) suspected (tir4) and 4 (16%) malignant (Figure-1). In all 14 nodules, that seemed benign in cytomorphology, the molecular dosage of the B-RAFV600E was negative (100%). All nodules were benign in the definitive histopathological examination (100%). In these cases, the surgical indication arises from the presence of multinodular goiter, sometimes with dysfunctional, compressive symptoms with lateral deviation and / or tracheal compression and / or mediastinic immersion. Of the four indefinite nodules, 1 (25%) was positive to the B-RAFV600E and 3 (75%) negative. The positive nodule at the molecular dosage of the B-RAFV600E proved to be a capsulated classical papillary carcinoma (100%), while the three B-RAFV600E, 2 (83.3%) were benign, while 1 (17.7%) was a follicular variant of papillary carcinoma. Of the three nodules whose cytomorphology appeared suspect, 1 (20%) was positive for the molecular dosage of the B-RAFV600E, while 2 (80%) were negative. At histopathological examination, the positive B-RAFV600E nodule resulted in papillary carcinoma (100%), while the 2 negative B-RAFV600E nodules were benign (a hyperplastic nodule, a follicular adenoma). Of the 4 nodules that appeared cytomorphological malignant, 3 (87.5%) were positive BRAF V600E and 1 (12.5%) BRAFV600E negative. The 3 positive nodules at the B-RAFV600E were papillary carcinomas, 2 of which were classics and 1 tall-cell variant. The negative B-RAFV600E nodule, instead, resulted in follicular variant papillary carcinoma (Table-1. Figure-2). In the control group, instead, the results were as follows: 15 (60%) appeared benign (tir2), 3 (12%) undetermined (tir3), 4 (14%) suspected (tir4) and 3 (14%) malignant (tir5). The definitive histopathological examination confirmed the benign nature of all 15 nodules that appeared like that at cytomorphologic examination

(100%). Of the 3 nodules indeterminate, 1 (16.67%) resulted in papillary carcinoma and 2 (83.37%) of benign nature. Of the 3 suspected nodules, 1 (33.3%) was a follicular variant of papillary carcinoma, 1 (33.3%) follicular carcinoma, 1 (33.3%) follicular adenoma. The 3 malignant nodules, on the other hand, were all papillary carcinoma (100%), of which 2 were classical, 1 follicular variant (Table-2). In the experimental group, considering the determination of the B-RAFV600E and referring to histopathological findings of benignity and malignancy, the presence of 5 true positive (BRAFV600E related to carcinoma) and 1 false negative was found, while the real negative were 19. On the entire sample of 25 patients, therefore, the sensitivity of the test was 83.3%, while the specificity was 100 %. The calculation of positive predictivity, given the VP / (VP + FP) formula, shows a positive prediction of 100, while the negative predictivity, given by the VN / (FN + VN) ratio, was 95 %. Diagnostic accuracy, expressed as VP + VN / TOT, was 96 %.

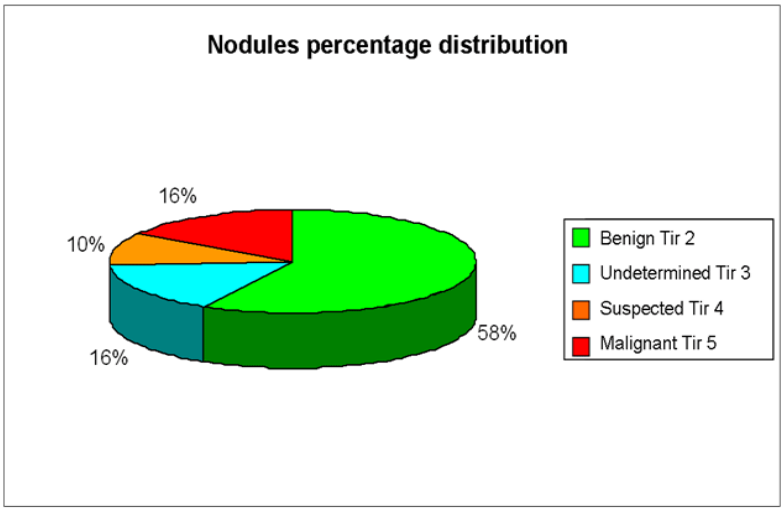

Figure 1: Percentage distribution of nodules in the experimental group

Table 1: Experimental group results

Fine needle aspiration cytology (FNAC)	Nodules number	Presence of $BRAF^{V600E}$ mutation	Histological examination
Tir 2	14 nodules	14 negative	14 benign
Tir 3	4 nodules	1 positive 3 negative	1 $BRA\text{-}F^{V600E}$ positive:-1 papillary carcinoma 3 $BRA\text{-}F^{V600E}$ negative:- 2 benign -1 papillary carcinoma follicular variant
Tir 4	3 nodules	1 positive 2 negative	1 $BRA\text{-}F^{V600E}$ positive:-1 papillary carcinoma 2 $BRA\text{-}F^{V600E}$ negative:-2 benign
Tir 5	4 nodules	3 positive 1 negative	3 $BRA\text{-}F^{V600E}$ positive:-3 papillary carcinoma 1 $BRA\text{-}F^{V600E}$ negative:-1 papillary carcinoma follicular variant

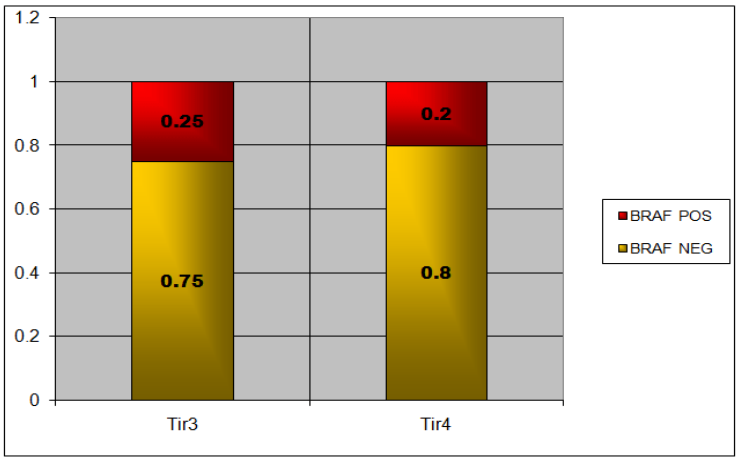

Figure 2: Percentage distribution of the positivity to BRAF mutation for tir 3 and tir 4

Table 2: Control group results

Fine needle aspiration cytology (FNAC)	Nodules number	Histological examination
Tir 2	15 nodules	15 benign
Tir 3	3 nodules	1 papillary carcinoma 2 benign
Tir 4	4 nodules	1 papillary carcinoma follicular variant 1 papillary carcinoma 1 benign 1 hyperplastic nodule
Tir 5	3 nodules	3 papillary carcinomas

Discussion

Comprehensive data analysis requires groups to be evaluated according to diagnostic categories. When the cytomorphological examination suggests a benign repertoire (Tir2), the B-RAFV600E determination, which is always negative, adds nothing to the diagnosis. When, however, the cytomorphological find was found suggestive of malignant lesion (Tir5) histological examination has always confirmed the diagnosis of nodule malignancy, with 100% concordance. In the experimental group, based on the only determination of the B-RAFV600E, a false negative was found. Thus, it is clear that the determination of the B-RAFV600E is superfluous both when the cytomorphological examination poses for a benign lesion and when it poses for a malignant lesion. In such cases, its execution only adds costs without adding any diagnostic aid and may be misleading for possible falsely negative results. The opposite is the discourse when the cytomorphology is not conductive and it allows nothing but a diagnosis of indefinite nodule (Tir3) or suspect nodule (Tir4).

In these cases, the therapeutic options may be different, in the case of an indefinite nodule, the surgical indication is relative: you can adopt a very attentive attitude, repeating the needle after six months, or a more aggressive attitude, pointing to the surgical intervention. In this case, however, there are extremely controversial scientific opinions about the extension of surgical exeresis when there are no other reasons indicative for total Thyroidectomy: in cases of single nodule, in fact, there are those who, however, wish to perform a total Thyroidectomy and who, on the basis of a probabilistic benignity criterion, propose in a first instance loboistemectomy, reserving the totalization intervention at a later stage, when cancer is diagnosed with histopathology .

In the case of suspected nodular lesions (Tir4), the surgical indication is absolute, even in this case; however, opinions about the extension of exeresis are controversial, with the same considerations being discussed for indefinite nodules (Tir3). In these two diagnostic categories then the determination of the B-RAFV600E seems to be overwhelming, in fact, while considering the relative size of the experimental samples tested, the sensitivity of the test is 83.3%, since false positives are possible, while its specificity is 100%, since no false positives have been found. Test sensitivity, unfortunately, is not 100%, because in thyroid cancers the B-RAFV600E mutation is not always present. As a characteristic of the genetic base of each cancer, in fact it is necessary a mosaic of mutations of proto-oncogens, onco-suppressor genes, pro-apoptotic genes, and anti-apoptotic genes, which contribute to alter the cell cycle regulation to the point of bringing out the malignant neoplastic phenotype. In this mosaic the B-RAFV600 mutation is not always present, it is absent in follicular carcinomas, and within papilliferous tends to prevail in classical and high-cell variants, generally lacking in the other. In spite of this, its specificity is 100%, therefore, in the face of a positive molecular assay, we have a diagnostic orientation of

certainty towards a papillary carcinoma lesion ; this data, in the face of a cytomorphologic diagnosis of indefinite nodule, renders the surgical indication absolute, it also makes it vanish the probabilistic "benignity" criterion on which the current diatribe is based on the extension of surgical exeresis , which also involves cytomorphologic ally suspect nodules (Tir4) .

Consideration should now be given to the therapeutic management of micro carcinoma. In these cases there are two currents of thought: one in favor of lobectomy, the other in favor of total Thyroidectomy. Those claiming for total Thyroidectomy are in favor of this treatment basically for two reasons: on the one side that of multicentricity-multifocality of cancer, whether for polyclonal origin, whether for intra-glandular metastasis, and on the other side that the follow-up after total Thyroidectomy is easier (dosage of serum Thyroglobulin and total body scintigraphy) allowing an earlier diagnosis of a possible recurrence of the disease. In this regard, the pre-operative diagnosis of B-RAFV600E molecular assay positivity has a particular meaning, although this mutation is more frequently present in classical papillary carcinoma, which is considered a favorable histotype , it is also frequently present in the high-cell variant, which has a worse prognosis. Regardless of the histological variant, it is still demonstrated that the expression of the B-RAFV600E mutation correlates with greater biological aggressiveness of the neoplasia, resulting in extra capsular extension with or without invasion of the nearby structures and regardless of size, local-regional lymph node metastasis, and relapse of the disease, all with a higher and statistically significant frequency than the negative B-RAFV600E neoplasm's. Based on this data it is thus understood how the positivity of the molecular dosage of the B-RAFV600E can be of fundamental help not only in the diagnostic definition but also in the choice of the surgical strategy regarding the extension of the exegesis and in the post-operative, both with regard to the possibility of relapse of disease at a distance of time both with regard radioiodine sensitivity therapy that is reduced in PTC expressing the B-RAF V600E mutation.

Conclusions

Our study shows that the molecular dosage of the BRAFV600E shows a diagnostic sensitivity for papillary carcinoma of 83.3%, but with a specificity of 100%. Its positive prediction is 100%. The data analysis has shown that its execution is superfluous when cytomorphological examination depicts the characteristics of benignity (Tir 2) or malignant lesions (Tir 5), resulting in only a rise in costs without adding anything to diagnostic definition. It is of great help in cases of indeterminate nodule (Tir 3) or suspicion (Tir 4), because although its negativity cannot exclude a cancer, its positivity will allow its diagnosis of certainty. This implies a fundamental improvement in diagnostic accuracy, dissipating the uncertainties of the cytomorphological examination and allowing to start the patient at the most appropriate surgical intervention and the most appropriate postoperative therapy. Based on this scientific evidence, we can conclude that the molecular dosage of the BRAFV600E should not be performed in cases where the cytomorphologic examination lays for a possibly benign (Tir 2) or malignant (Tir 5) injury but should be performed routinely when there is an indeterminate cytopathological picture (Tir 3) or suspicion (Tir 4).

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