The role of BRAF(V600E) mutation as poor prognostic factor for the outcome of patients with intrathyroid papillary thyroid carcinoma

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ABSTRACT

Background: BRAF(V600E) mutation, which represents the most frequent genetic mutation in papillary thyroid carcinoma (PTC), is widely considered to have an adverse outcome on PTC outcome, however its real predictive value is not still well stated. The aim of the present study was to evaluate if BRAF(V600E) mutation could be useful to identify patients with intrathyroid ultrasound-N0 PTC those who require more aggressive treatment, by central neck node dissection (CLND) or subsequent postoperative 131I treatment.

Methods: Among the whole series of 931 consecutive PTC patients operated on at 2nd Clinical Surgery of University of Padova and at General Surgery Department of University of Trieste during a period from January 2007 to December 2012, we selected 226 patients with an intrathyroid tumor and no metasteses (preoperetative staging T1–T2, N0, M0). BRAF(V600E) mutation was evaluated by PCR-single-strand conformation polymorphism analysis and direct genomic sequencing. We analyzed the correlation between the presence/absence of the BRAF(V600E) mutation in the fine-needle aspiration (FNA) and the clinical-pathological features: age, gender, extension of surgery, node dissection, rate of cervical lymph node involvement, tumor size, TNM stage, variant of histotype, mono/plurifocality, association with lymphocitary chronic thyroiditis, radioactive iodine ablation doses, and outcome.

Results: The BRAF(V600E) mutation was present in 104 of 226 PTC patients (47.8%). BRAF(V600E) mutation correlated with multifocality, more aggressive variants, infiltration of the tumoral capsule, and greater tumor’s diameter. BRAF(V600E) mutation was the only poor prognostic factor in these patients.

Discussion: In our series, BRAF(V600E) mutation demonstrated to be an adverse prognostic factor indicating aggressiveness of disease and it could be useful in the management of low-risk PTC patients, as supplementary prognostic factor to assess the preoperative risk stratification with the aim to avoid unnecessary central neck node dissection (BRAF pos.) or to perform complementary 131I-therapy (BRAF neg.).

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1. Introduction

Thyroid cancer is the most common malignancy of the endocrine system and it is one of the fastest growing cancers diagnosed worldwide. Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer, accounting for approximately 80% of cases. Its incidence has nearly doubled over the last 30 years and is thought to be due in part to earlier diagnosis of subclinical disease, particularly the small PTC [1,2].

In the majority of cases PTC has an excellent prognosis with 5- and 10-year patient survival rates of 95% to 98% but there are reported a 10% of cases with worse prognosis [3].

Similar to other cancer types, thyroid cancer progression and dedifferentiation involves a number of genetic mutations. Alterations of several oncogenes are described in literature: PTC/RET rearrangement, mutation of NTRK, p53, PAX8-PPARγ and RAS [4–6].

BRAF is a serine threonine kinase that is translocated to the cell membrane after being bound and activated by RAS, which results in the phosphorylation and activation of mitogen activated protein kinase (MAPK) and other downstream targets of MAPK signaling pathway [7].

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The BRAF(V600E) mutation, a valine to glutamic acid substitution at position 600, is found in more than 50% of overall thyroid malignancies. BRAF mutation exists in PTC and PTC-derived anaplastic thyroid cancer and it has not been found in follicular thyroid cancer and medullary thyroid cancer [7–10].

BRAF mutation is the most common genetic alteration in thyroid cancer, particularly in PTC and it has been identified in approximately 29% to 69% of PTCs and in > 80% of PTCs of the tall cell variant, whereas they have not been detected in benign lesions or in the majority of those (80%) with the follicular variant of PTC [7–10]. The association of BRAF mutation with poor clinicopathological characteristics of PTC further demonstrated the importance of the BRAF mutation alternation in PTC [7–14].

Several authors stressed the role of BRAF analysis performed on cytologic material from fine-needle aspiration (FNA) of indeterminate nodules and of small papillary cancers in order to program the extension of surgery [15,16]. Other Authors [11] suggested the assessment of BRAF mutation in histological specimens to identify, among low-risk PTC patients, those who could avoid 131I treatment.

Our aim was to evaluate the impact of presence of BRAF mutation in the FNA in the outcome of patients with intrathyroid PTC to determine its real importance in the preoperative assessment of surgical treatment.

2. Materials and methods

Among 931 consecutive patients who underwent surgery for histologically proven PTC from January 2007 to January 2012 at 2nd Clinical Surgery, University of Padova and at Institution of General Surgery, University of Trieste, 226 patients with intrathyroid PTC, (preoperative stages I and II) without thyroid capsule infiltration or extrathyroid invasion, in absence of preoperative evidence of lymph nodes disease ultrasonographically classified N0, were included. The patients were divided into two groups: group A with BRAF mutation on the preoperative FNA and group B (control group) without BRAF mutation. We excluded patients with metastatic disease (M1), with R1 thyroidectomy, and patients who underwent concomitant lateral neck dissection.

The extent of surgery had not been planned on the basis of the presence of BRAF mutation.

A central neck dissection was performed on the basis of the neoplasm's diameter (> 1 cm) or on the basis of intraoperative recognition of suspected lymphadenopathy, thyroid capsular or extrathyroidal invasion.

Complete preoperative assessment (thyroid hormone serum levels, thyroglobulin and anti-thyroglobulin autoantibodies, ultrasonography [US] to evaluate both nodule size and gland volume, FNAC) was obtained from all patients. Preoperative informed consent was obtained from all patients.

Postoperative 131iodine treatment, TSH, thyroglobulin and anti-thyroglobulin autoantibodies were performed with the withdrawal of hormonal therapy or with the injection of exogenous rhTSH. Subsequently the patients were followed up by US, TSH, thyroglobulin and anti-thyroglobulin autoantibodies dosage.

We retrospectively reviewed clinical and histopathologic documents and compared among two groups these variables: age, gender, extension of surgery, node dissection, rate of cervical lymph node involvement, tumor size, TNM stage (7th edition, 2009) [17], mono-/plurifocality, extrathyroidal tumor extension, radioactive iodine treatment, postoperative complications, and outcome (metastasis, completeness-of-resection, and recurrence rates).

Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total</th>
<th>BRAF(V600E)</th>
<th>Without mutation</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>226</td>
<td>104 (46%)</td>
<td>122 (54%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>179</td>
<td>86 (42.7%)</td>
<td>93 (37.3%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>M</td>
<td>47</td>
<td>18 (38.3%)</td>
<td>29 (33.8%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Age</td>
<td>44 (8–78)</td>
<td>44 (17–77)</td>
<td>45 (8–78)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Variants types</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sclerosant</td>
<td>4</td>
<td>3 (2.88%)</td>
<td>1 (0.82%)</td>
<td>&lt;0.036</td>
</tr>
<tr>
<td>Tall cells</td>
<td>6</td>
<td>3 (2.88%)</td>
<td>3 (2.46%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Ossified</td>
<td>18</td>
<td>8 (7.7%)</td>
<td>10 (8.2%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Follicular</td>
<td>44</td>
<td>12 (11.53%)</td>
<td>32 (26.24%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Classic</td>
<td>78</td>
<td>75%</td>
<td>76 (62.32%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total thyroidectomy</td>
<td>212</td>
<td>100 (96%)</td>
<td>112 (92%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Emithyroidectomy</td>
<td>14</td>
<td>4 (4%)</td>
<td>10 (8%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>CLND</td>
<td>169 (74.7%)</td>
<td>81 (78%)</td>
<td>88 (72%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>TNM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>202</td>
<td>91 (45.3%)</td>
<td>111 (55.1%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>II</td>
<td>11</td>
<td>4 (3.85%)</td>
<td>7 (6.36%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>III</td>
<td>13</td>
<td>9 (7.3%)</td>
<td>4 (3.85%)</td>
<td>&lt;0.036</td>
</tr>
<tr>
<td>Capsular invasion</td>
<td>8 (7.7%)</td>
<td>2 (1.63%)</td>
<td>&lt;0.016</td>
<td></td>
</tr>
<tr>
<td>Monofocality</td>
<td>136</td>
<td>58 (55.77%)</td>
<td>78 (64.6%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Multifocality</td>
<td>90</td>
<td>46 (44.23%)</td>
<td>44 (36%)</td>
<td>&lt;0.026</td>
</tr>
<tr>
<td>N+</td>
<td>31</td>
<td>15 (44.2%)</td>
<td>16 (51.6%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Association with CLT</td>
<td>85</td>
<td>42 (7.13%)</td>
<td>43 (35.2%)</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>Tumor diameter in mm</td>
<td>11.9 (1–40)</td>
<td>13.5 (1–50)</td>
<td>11.3 (1–40)</td>
<td>&lt;0.048</td>
</tr>
<tr>
<td>Micro-PTC</td>
<td>91 (40%)</td>
<td>33 (31.7%)</td>
<td>58 (45.5%)</td>
<td>&lt;0.029</td>
</tr>
</tbody>
</table>

F: female; M: male; CLND: central neck node dissection; CLT: chronic lymphocytic thyroiditis; PTC: papillary thyroid carcinoma; N.S.: not significant.
2.1. DNA extraction and analysis

Genomic DNA was extracted from the FNA sample by using the QiAamp DNA micro Kit (Qiagen) according to the manufacturer’s protocol. Exon 15 of the BRAF gene (NM_004333.4) was amplified and then sequenced on an ABI PRISM 3130 genetic analyzer (Applied Biosystems). The PCR reaction protocol and primers are described elsewhere [18].

2.2. Statistical analysis

Comparison of categorical variables was performed by Chi²-statistic using the Fisher exact test when appropriate. Statistical analyses were performed using a statistical software package (Statistica 7) for Windows. All P values < .05 were considered significant.

3. Results

Totally 226 patients, (179 females and 47 males) were enrolled in this study whereas 705 patients were excluded because the preoperative stage was T3 or T4 or preoperative metastases were found. Follow-up evaluations were completed for 188 patients (83%). Mean follow-up time was 43 months (range 3–156), 70% of patients had a follow-up longer than 3 years.

The results are summarized in Table 1 and Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Follow-up</th>
<th>188</th>
<th>90</th>
<th>98</th>
<th>N.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of follow-up (months; mean, range)</td>
<td>43 (1–156)</td>
<td>39 (1–343)</td>
<td>45 (4–156)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>131 radiotherapy</td>
<td>146</td>
<td>72</td>
<td>Ablative therapy = 69</td>
<td>74</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ablative therapy = 69</td>
<td></td>
<td>N.S.</td>
</tr>
<tr>
<td>Administered activity</td>
<td></td>
<td></td>
<td>Therapeutic therapy = 7</td>
<td></td>
<td>N.S.</td>
</tr>
<tr>
<td>Number of radioiodine treatments. Mean, range</td>
<td>1.03 (1–3)</td>
<td>1.09 (1–3)</td>
<td>1.02 (1–2)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Tg serum levels ([ng/dL], mean, range)</td>
<td>2.7 (range, 0.1–136.9)</td>
<td>2.5 (0.1–136.9)</td>
<td>2.4 (range, 0.1–58)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Disease free</td>
<td>188</td>
<td>90</td>
<td>98</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Interval free survival</td>
<td>16 months</td>
<td>16 months</td>
<td>16 months</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Relapse of disease</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Re-operation</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Not disease free</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Deceased for other causes</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N.S.</td>
<td></td>
</tr>
</tbody>
</table>

N.S.: not significant.

(Table 1) [P < 0.036]. There were reported 8 cases (7.7%) and 2 cases (1.63%) respectively of capsular infiltration in group A and B [P < 0.016]. There were 46 (44.23%) pluri-focality in group A and 44 (36%) in group B, respectively [P < 0.026]. The association with lymphocitary chronic thyroiditis was major in group A than in group B (7.13% vs. 35.2%) [P < 0.016].

Neck recurrence occurred in 3 patients (2.88%) in group A and among these two patients (postoperative stage III and stage I, respectively) needed a subsequent lymph nodes lateral neck dissection, whereas another patient underwent to a further therapeutic radioiodine treatment. All these patients, at the moment, are disease free.

On the other hand, in group B, only one patient (stage I) presented relapse disease at 12 months after surgery and underwent to lateral node dissection (0.82%) [P = N.S.] and now is alive without any sign of disease relapse.

Mean serum postablative Tg levels after levothyroxine withdrawal resulted a little higher in group A than group B (2.5 ng/mL vs. 2.4 ng/mL) [P = N.S].

The 131I therapy was performed in 146 patients (77%), at therapeutic doses in 12 (8.2%) and at ablative doses in 134 (91.8%).

Comparing group A vs. group b the 131I therapy was performed in 72 (80%) vs. 74 (75%). The 131I therapy was performed at therapeutic doses in 7 (9.72%) (for node metastases in 2, for thyroidal remnant tissue in 5) vs. 5 (6.76%) (for node metastases in 2, for thyroidal remnant tissue in 3) patients and at ablative doses (< 150 mCi) in 65 (90.28%) vs. 69 (93.24%) patients (Table 2).

The mean radioactive iodine ablation doses were 132.63 mCi in group A vs. 129.42 mCi in group B [P = N.S].

4. Discussion

The rapidly rising incidence of thyroid cancer in general and in PTC may be due to increased use of ultrasound examination in routine thyroid investigation that allowed the easier and more frequent identification of subclinical disease with increased diagnosis of small PTC [1,2,8].

Several authors demonstrated that the excessive activation of BRAF/MAPK signaling pathway due to BRAF mutation plays a central role in the tumorigenesis and development of PTC [10,19].

BRAF mutation also predisposes tumors to dedifferentiation and to progression toward poorly differentiated and anaplastic forms, which explain the unfavorable prognosis of these patients [20].

Recent studies have showed that BRAF(V600E) mutation in thyroid cancer appears to be associated with a poorer outcome,

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high risk clinicopathological characteristics, extrathyroidal invasion, lymph nodal metastasis, advanced TNM stage tumor, recurrence and reduced sensitivity of radiiodine therapy [21–24]. Thus, induced several authors to analyze the prognostic factor of BRAF mutation in FNA to assess the preoperative risk stratification of thyroid lesions [15,16] and in histological specimens to evaluate its prognostic significance [11].

The above aggressive features of PTC were also confirmed to correlate independently with BRAF mutation when general data of patients such as age, gender, and residence and tumor size, multifocality, and histology subtype were adjusted for multivariate logistic regression analysis [11]. However, a number of studies showed conflicting results that demonstrated there was no association between BRAF mutation and poor clinicopathological factors [25,26].

Detection of BRAF mutation on fine-needle aspiration cytology was utilized to predict surgical strategy, more aggressive in patients with the V600E positivity.

The BRAF (V600E) mutation assessment on the cytological examination has been employed, in the management of thyroid indeterminate nodules (Thy 3), to evaluate which of these needed the surgical resection or decide whether to perform respectively a total thyroidectomy or hemithyroidectomy if the BRAF mutation was present or not.

Regarding thyroid nodules suspicious (Thy 4) or malignant (Thy 5), the presence of the BRAF mutation in the cytological examination, in the reason of its high prognostic negative value, guided some authors [16] to decide if perform, in association to total thyroidectomy at least CLND.

Regarding the micro-PTC (< 1 cm of diameter) [27], which habitually has a satisfactory prognosis, BRAF mutation may be a more important indicator for high risk of poor clinicopathological features and progression to advanced stage.

The clinical management of patients with small PTC and with micro-PTC remains unclear, because these tumors generally are considered clinically indolent with a good prognosis, although some of them may exhibit aggressive clinical behavior [27,28].

In fact, generally, PTC has a high cure rate with 10-year survival rates estimated at 80% to 90% after appropriate treatment including surgical procedure and radiiodine therapy. However, cervical metastases are present in about 30% of small papillary carcinomas and in more than 50% of the larger tumors. Moreover, recurrence rate of differentiated thyroid cancer is estimated up to 30% and cancer death rate as 8% after initial treatment at 30 years of follow-up are reported [27,29].

Some Authors proposed prophylactic central neck dissection in patients with BRAF mutation analysis performed on cytological material [16]. In agreement with data from the literature, we observed that BRAF mutation was present in 46% of patients. Considering the main prognostic factors in the thyroid cancer, in our cohort patients, there was not present a statistically significant difference regarding gender an age of the patients studied. On the contrary, according with other studies [29], follicular variant of PTC (FVPCs) had a low rate of BRAF mutations (11.53%) and, on the other hand, that this molecular alteration was detected in 75% of classic PTC. In particular, regarding the sclerosant variant of PTC, which constitutes an aggressive variant of PTC, a BRAF mutation was present in 2,88% of patients. This is a low percentage but it resulted statistically different if compared with the patients without BRAF mutation where this variant were present in only one patient (0.82%).

In our series, although BRAF(V600E) mutation was associated to more advanced stage (P = 0.036), greater diameter of tumor with a minor rate of micro-PTC, major capsular invasion, and multifocality none differences in the follow-up was registered. Our data, seems reflect a low influence of BRAF mutation in the prognosis of PTC, demonstrating that BRAF mutation seems to be a poor prognostic factor for the aggressiveness of the disease, but it not resulted independent from other clinical-pathological features in low-risk intrathyroid PTC patients.

Another important aspect emerged analyzing our data, reaffirming the poorer prognosis in the BRAF population, is that the patients who presented the BRAF mutation on FNA had a minor association with chronic lymphocytic thyroiditis (CLT), thus reflects the probable protective role of CLT hypotized by some authors [30] in fact several studies described the better prognosis of PTC associated to CLT.

5. Conclusions

In our experience, the BRAF(V600E) mutation demonstrated to be correlated with the aggressiveness of disease but not with persistence of disease. Furthermore, in our low-risk intrathyroid PTC patients groups, the BRAF(V600E) mutation not resulted an independent poor prognostic factor from other clinical-pathological features. At least, it could be useful to search for the BRAF(V600E) mutation in the workup of low-risk PTC patients as supplementary prognostic factor to assess the preoperative risk stratification of thyroid lesions and to distinguish those who require a stricter follow-up, more aggressive management as more extent surgery or postoperative [31] treatment.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References


