Application of Molecular Diagnostics to the Evaluation of the Surgical Approach to Thyroid Cancer

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Abstract: Recent important studies that include long-term follow-up have shown that BRAF and RAS mutations can have negative implications for disease recurrence and survival. BRAF positivity has been shown to be associated with decreased survival and is an independent predictor of poor prognosis. Reliable pre-operative identification of high-risk papillary thyroid cancer (PTC) patients may productively guide initial surgical management since reoperative neck surgery is associated with increased morbidity. However, it is probably too early to conclude that at present it is possible to tailor surgical therapy patient by patient only on the basis of their mutational status. Other important parameters, not including molecular testing, represented by some specific morphological aspects, still play an important role, probably still more significant than molecular diagnostics, such as neck ultrasonography. Pre-operative knowledge of BRAF-positive PTC could alter the initial surgical treatment for at least 20% of patients and can potentially prevent the increased morbidity associated with reoperative neck exploration. However, additional multi-institutional and randomized studies will be needed to further define the role of the pre-operative identification of BRAF positivity to guide not only the initial extent of total thyroidectomy (TT) but also the need for and extent of lymphadenectomy.

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INTRODUCTION

Thyroid cancer is the 7th most common cause of any new malignancy in the US for women, and the 14th in men [1, 2]. While the yearly incidence of thyroid cancer (ThyCa) has been growing in the last decades, the percentage of cancer deaths *per* year, relative to the number of new cases, has decreased [1, 2]. The histotype of ThyCa is papillary (PTC) in 79% of cases, and follicular in 13%, medullary in 3%, and anaplastic (ATC) in 2% [3]. PTC presents most commonly between 30 and 50 years of age, with a preponderance in female gender (female/male=3:1). The radiation exposure is a risk factor of PTC [4]. The increasing incidence of ThyCa is associated with a higher number of advanced disease characterized by the loss of cancer differentiation and metastatic spread [1], causing high morbidity, but not necessarily death [5].

The knowledge of the molecular pathways involved in the pathogenesis of ThyCa has made possible the development of new therapeutic drugs able to block the oncogenic kinases (BRAF V600E, RET/PTC) or signaling kinases (vascular endothelial growth factor receptor, platelet-derived growth factor receptors) involved in cellular growth and proliferation [6-9].

However, molecular diagnostics have been also advocated as an important tool in order to personalize the surgical approach to patients presenting with ThyCa. The advantage of such an investigation in fact is the possibility of having this molecular testing available before surgery. In case the presence of a mutation is revealed to consistently modify the tumor's prognosis, it would be possible to tailor the surgical strategy accordingly. In particular it seems that small PTC which are currently classified as "low risk" tumors, because of their limited size and well differentiated histotype, could take the greatest advantage from such pre-operative analysis.

In PTC, RET/PTC rearrangements are found in 30-40%, RAS mutations in about 10%, and BRAF mutations in approximately 40-50% of cases, with no overlap among these mutations; a higher prevalence of BRAF mutations (up to 70%) has been observed in dedifferentiated PTC [10]. BRAF mutation in PTC has been independently associated with the absence of tumor capsule and tumor iodine (131-I) avidity, tumor recurrence, and treatment failure of recurrent disease [11]. RAS mutations are also found in ~35% of poorly differentiated and ~50% of ATCs, where the presence of RAS mutations seems to correlate with more aggressive tumor behaviour and poor prognosis [12].

For the above mentioned reasons, BRAF and RAS [13-15] seem to play an important role in modifying the prognosis of these tumors. When available on a cytological specimen before surgery, they could lead the surgeon to modify his surgical strategy in two important points:

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- Opportunity of performing a central compartment lymphadenectomy also in PTC which have been classified as “low risk” according to conventional staging and where pre-operative imaging did not show any involvement of these lymph nodes.
- Reducing the necessity of performing a completion thyroidectomy following a unilateral surgery where final histology has shown a PTC in a patient with an undetermined nodule (Thy 3).

Actually, an important indication for surgery is greatly represented by PTC less than 1 cm size (micropapillary carcinomas). Despite very low mortality associated with micropapillary thyroid cancer, locoregional recurrence is common and controversy exists regarding optimal surgical treatment and the role of adjunctive radioiodine. A study suggested that patients with micropapillary multifocal disease have a reduced risk of recurrence after a total (T)/near-total thyroidectomy (NTT) compared with less surgery [16].

However, molecular diagnostic could help in the choice of the correct surgical approach in these patients. In fact, using laser dissection, the BRAF mutation can be found even in microcarcinomas as small as 1 mm or less [17].

Moreover, a more accurate stratification according to the mutational status could also modify the follow-up of these patients, for example avoiding unnecessary radioiodine therapy after surgery.

Here we review the use of pre-operative molecular diagnostic for BRAF or RAS mutations in order to personalize the surgical approach to patients presenting with a ThyCa.

BRAF MUTATION

Mutations in the BRAF gene, a member of the RAF family protein which binds RAS, lead to a constitutional phosphorylation of MEK and, in turn, of MAPK pathways. The exon 15 V600E mutation (T1799A) represents >90% of BRAF mutations and is found in about a half of PTC (45%). The presence of BRAF V600E has been independently associated with recurrent disease, with the absence of tumor capsule and with the loss of 131-I avidity [11, 18]. Other BRAF activating point mutations have been described in other positions (for instance, 599 and 601), but their prevalence is definitely lower than in 600 [19, 20]. Recently, targeted therapies against BRAF have been developed [9, 21].

A large study correlated the BRAF V600E mutation with both clinical-pathological features and the degree of neoplastic infiltration to redefine the reliability of the actual system of risk stratification in a large selected group of PTCs smaller than 20 mm. The presence of BRAF mutations was examined in 1060 PTCs less than 20 mm. The overall frequency of the BRAF V600E mutation was 44.6%. BRAF mutations showed a strong association with PTC variants (classical and tall cell), tumor size (11-20 mm), multifocality, absence of tumor capsule, extrathyroidal extension and lymph node metastasis [22].

This paper clearly demonstrates that many parameters linked to a more severe prognosis were clearly represented in the presence of BRAF mutation. Some of them of course could modify the surgical approach to the patients since they

could involve basically two aspects of the operation: the extent of the thyroidectomy and the lymph node invasion (Fig. 1). For this reason it would be particularly important to have this information available before surgery: in fact, the prevalence of capsular invasion and lymph node metastases (LNMs), possibly increased when BRAF mutation is present, could lead the surgeon to adopt a more aggressive approach [23]. The possibility of a capsular invasion from the tumor in fact makes the tumor behaving as a T3 in spite of its limited size and so would not leave any space for an operation less than a TT. Furthermore, the highest caution should be used also with regard to the structures which could be involved by the tumor invasion: in particular, the trachea for those cancers located in the posterior part of the gland and the strap muscles for those located in the anterior part. Apparently, also the multifocality of the tumor would be an important argument in favor of a TT, but the probability of a multifocal tumor in the presence of BRAF mutation is much lower [22]. So, it is not surprising that still some Authors propose a hemithyroidectomy for small PTC, in particular, if pre-operative ultrasonography does not show any nodularity in the contralateral lobe [24].

In terms of the probability of leaving a tumor behind then, we can agree that the presence of the mutation is not an important obstacle towards a more limited surgery, but we should not disregard the general behavior of this kind of tumors that seem to have a worse prognosis [25] and as consequence could see the possibility of a further treatment with radioiodine strongly limited by an operation such as a simple lobectomy.

Another important issue for surgery would be the ability of knowing the lymph node status at the central compartment level before deciding the operative strategy: this problem has been widely debated since pre-operative imaging generally does not give a reliable information about the presence of LNMs in this area and for this reason, the VIth level lymphadenectomy is often proposed as a prophylactic rather than therapeutic intervention. Undoubtedly, an information other than imaging [26], available before operation and able to suggest the possible presence of LNMs in that district, would be crucial for a prompt and timely surgical planning. Early reports in literature [27, 28] had raised a great enthusiasm about the significant role of BRAF in influencing the prognosis of PTC and the mutation seemed to play a strategic role in predicting lymph node involvement in those tumors [29].

In a first study [28] the clinical, cytologic, and pathologic parameters of 106 consecutive surgically treated patients with BRAF-positive PTC were compared with a concurrent cohort of 100 patients with BRAF-negative PTC. In PTC, BRAF mutations were associated with cervical recurrence and with reoperation. The Authors suggested that pre-operative cytologic identification of BRAF mutation has high specificity and may guide the initial extent of thyroidectomy and node dissection.

More recently, a study investigated whether pre-operative BRAF analysis may assist determination of surgical extent, including prophylactic central lymph node dissection (CLND) with variable clinicopathological risk factors for central lymph node metastasis, in patients with PTC [29].

Fig. (1). The presence of BRAF mutation could modify the surgical approach to the patient, since it could involve basically two aspects of the operation: the extent of the thyroidectomy and lymphadenectomy.

One hundred forty-eight PTC patients were prospectively enrolled with clinically node-negative neck who received a TT and prophylactic CLND. BRAF mutation was tested on pre-operative fine-needle aspiration biopsy (FNAB) specimens. The prevalence of the BRAF V600E mutation was 53.4%, and the rate of occult central lymph node metastasis was 25.7%. In a multivariate analysis, patients with pre-operative positive BRAF mutation were significantly more likely to have occult central lymph node metastasis. The Authors suggest that pre-operative BRAF analysis by FNAB may assist in predicting occult central lymph node metastasis in patients with PTC and clinically node-negative neck.

In the study by Basolo *et al.* [22] though the frequency of capsular invasion and lymph node involvement in the presence of the mutation were already compared, despite a similar statistical significance for both parameters, it was clear that a different odd ratio (3.74 vs 3.20) able to demonstrate the importance of lymph node involvement had to be reconsidered: this is what was highlighted by several following studies [30, 31] which showed a remarkable skepticism about the role of BRAF mutation in determining a significant increase in the invasion of lymph nodes by the tumor.

Gandolfi *et al.* [30] evaluated the presence and allele percentage of the BRAF in 132 cases of well-differentiated PTCs with ($n = 37$) or without LNMs ($n = 95$) and in 40 LNMs matched with 35 PTCs. No significant differences were observed in either the occurrence or the allele percentage of V600E mutation between the 2 groups of PTCs with or without LNMs.

A prospective study evaluated the correlation between the BRAF mutation and lymph node metastasis [31]. A total of 51 patients with PTC underwent TT and routine prophylactic CLND. All patients were tested for the BRAF mutation. Results: Positive lymph nodes were found in 54.9% of patients. The BRAF mutation was found in 15 patients (29%). BRAF was not correlated with LNMs. The Authors suggest that testing for the BRAF mutation does not help in deciding whether or not to perform CLND.

It seems more reasonable to see the problem of the relationship between lymphatic involvement and BRAF in a different prospective. Even though we can share the opinion of a certain predictive power of the mutation in determining the lymph node status, the problem must be seen in a more general context where also other morphological conventional parameters still result as prominent prognostic factors [32]. Among these important pathological parameters, the surgeon should not ignore some aggressive histological variants and the capsular invasion that might be excluded also after a frozen section. Matching this kind of morphological information with molecular testing should give a better overview when a decision has to be made either before or during surgery.

RAS MUTATION

RAS ("Rat sarcoma") gene family encodes G-proteins that activate MAPK and PI3K/AKT pathways. Point mutations of N-RAS and H-RAS at codon 61 and K-RAS at codon 12 or 13 are the most common [33].

RAS mutations are not very common in PTCs accounting for not more than 10-15% of the cases, but this value rises significantly in the follicular variant of papillary cancer (FVPC) where BRAF by opposite is very poorly represented. Furthermore it is present in a significant number of the patients with a follicular carcinoma where it can reach values as high as 40-50% of the total [12, 34].

RAS mutations correlate with tumor aggressiveness and they are found effectively in half of anaplastic cancers and poorly differentiated cancers [12, 33].

In a first study the expression of p21, the ras encoded protein, was evaluated in primary tumor of 45 patients with PTC. The combination of p21 expression and age $\geq$ 40 was a prognostic indicator of poor outcome [35].

In a second study, tumors of 91 patients with papillary carcinoma were studied. Multivariate analyses showed that positive RAS mutation and age were two independent prognostic factors for prediction of death from papillary cancer and recurrence of cancer [36].

In our Group it was verified that in a cohort of FVPC patients presenting only an intrathyroidal extension, 61 out of 215 showed a RAS mutation (28.4%) whereas among 9 patients with an extra thyroidal extension, the same mutation was never present (unpublished data).

An important consequence emerging from these data is the importance of taking into account also RAS mutation, in particular N-RAS mutation, when operating a patient with a FVPC. Since this specific mutation seems to correlate negatively with tumor aggressiveness, the surgical therapy might be tailored accordingly: in particular, even if the mutation has been found in the histological specimen after surgery, in all those cases where hemithyroidectomy has been performed for an undetermined nodule (Thy 3) and the final diagnosis is FVPC, a completion thyroidectomy might be avoided with an acceptable risk margin.

Evidently, the possible applications of the mutation status vary widely according to the time when the molecular testing is available: in other words whether it was provided before surgery on a cytological aspiration or after surgery on the histological specimen. In the first case the surgeon should reasonably decide to perform a TT in every case when the molecular testing addresses a more aggressive form; the problem whether associating to thyroidectomy a central compartment clearance or not is more complex and, considering the most recent literature [30, 31], it is difficult to completely agree with the indication by Yip *et al.* [28] about the necessity of a VIth level lymphadenectomy in all the cases of PTC presenting with a mutated BRAF. We would rather suggest to perform it only in selected cases where other conventional parameters suggest a negative prognosis: in this case it could be advisable even if pre-operative imaging is negative at this level. Hemithyroidectomy is not advisable in these cases but it would be a correct surgical indication if the BRAF analysis shows a wild type and the tumor is of limited size (less than 2 cm) and with a normal contralateral lobe.

If the molecular testing is available after surgery and the operation consists of a hemithyroidectomy, the decision might be different in case BRAF is mutated, because a com-

plete thyroidectomy would be highly advisable, whereas the presence of N-RAS mutation in a FVPC would suggest to avoid a second surgery because a simple lobectomy would not expose the patient to a significant risk.

CONCLUSION

Recent important studies that include long-term follow-up have also shown that BRAF mutations can have negative implications for disease recurrence and survival [37]. BRAF positivity was associated with decreased survival and was an independent predictor of poor prognosis. Reliable pre-operative identification of high-risk PTC patients may productively guide initial surgical management since reoperative neck surgery is associated with increased morbidity [38, 39].

However, it is probably too early to conclude that at present it is possible to tailor surgical therapy patient by patient only on the basis of their mutational status [40, 41]. Other important parameters not consisting of molecular testing, represented by some specific morphological aspects still play an important role, probably still more significant than molecular diagnostics [42].

A pre-operative neck ultrasound has been described to have high sensitivity (83%) and specificity (93%) for the detection of thyroid cancer metastatic to cervical lymph nodes [26, 43, 44].

Other series demonstrated lower efficacy, with an overall sensitivity of 76% and specificity of 65% for pre-operative ultrasound identification of any (central or lateral) cervical lymph node metastasis [28].

Ultrasound is known to be observer dependent, and different experiences with lymph node mapping techniques have been variable. It is also possible that nodal micrometastases that do not have suspicious ultrasound findings could contribute to the observed findings.

Missed metastatic disease in lateral lymph nodes, however, may contribute to a high reoperative rate in BRAF-positive PTC patients. Pre-operative ultrasound to characterize lateral compartment lymph nodes can be helpful in guiding the extent of lymphadenectomy and should be performed routinely, especially in patients with BRAF-positive PTC.

The pre-operative identification of BRAF positivity may guide not only the initial extent of TT but also the need for and extent of lymphadenectomy. The poor prognosis of BRAF-positive PTC described in the literature is thought to be partly caused by radioactive iodine resistance [45].

Because BRAF-positive PTC may be refractory to conventional adjuvant thyroid cancer therapies, optimizing initial surgery may be the best therapeutic option. Some suggest routine central compartment lymphadenectomy for all patients known to have PTC preoperatively, but the issue is still controversial. Pre-operative identification of BRAF positivity could therefore be important in differentiating patients who may benefit from thorough bilateral central compartment dissection.

A better implementation of these new techniques would be advisable, integrating them into more conventional patho-

logic parameters. This could probably allow for more individualized surgical procedures. In particular, also the necessity for a post-operative radioiodine therapy should be reconsidered in the light of these new technologies in order to tune it in accordance to a new and more individualized risk stratification. Certainly, a reduction in the number of unnecessary completion thyroidectomies would be possible using molecular diagnostics [46]. A risk which should not be undervalued is that, after an initial and in some cases justified enthusiasm, these techniques might be partly disregarded because of their complexity and high costs not paralleled by an equivalent efficacy; this already happened to BRAF obtained on cytological specimens as a diagnostic tool in discriminating between benign and malignant tumors inside the wide class of undetermined nodules before surgery.

In conclusion, pre-operative knowledge of BRAF-positive PTC could alter the initial surgical treatment for at least 20% of patients and can potentially prevent the increased morbidity associated with reoperative neck exploration. However, additional multi-institutional and randomized studies will be needed to further define the role of the pre-operative identification of BRAF positivity to guide not only the initial extent of TT but also the need for and extent of lymphadenectomy.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

ATC	=	Anaplastic thyroid cancer
CLND	=	Central lymph node dissection
FNAB	=	Fine-needle aspiration biopsy
FVPC	=	Follicular variant of papillary cancer
LNMs	=	Lymph node metastases
NTT	=	Near-total thyroidectomy
PTC	=	Papillary thyroid cancer
ThyCa	=	Thyroid cancer
TT	=	Total thyroidectomy

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