FNA Cytopathology and Molecular Test Characteristics in the Changing Landscape of Papillary Thyroid Carcinoma

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Abstract

While the reported “incidence” of thyroid cancer is increasing, the mortality from thyroid cancer has remained flat. The implication is that most of the newly discovered cancers represent indolent disease. A group of oncologists advocate change to the cancer terminology for indolent neoplasms, reserving the term “cancer” or “carcinoma” for those tumors that would have a reasonable likelihood of lethal behavior if untreated. Nonetheless, a minority of thyroid carcinomas is potentially aggressive and the dilemma in thyroid nodule diagnostics and management is the prevention of morbidity and mortality while minimizing the surgical overtreatment. Over the recent decades, papillary thyroid carcinoma (PTC) has increased in incidence largely in part due to the rise of the follicular variant papillary thyroid carcinoma (FVPTC) while follicular carcinoma has become a rare entity. Extensive studies in this area have led to further subclassification of FVPTC with clinicopathologic differences: 1, non-invasive encapsulated FVPTC; 2, invasive encapsulated FVPTC; and 3, infiltrative FVPTC. Among these subtypes, non-invasive encapsulated FVPTC is considered a potentially aggressive PTC with recurrence rate of 16% and mortality of 12%. Infiltrative FVPTC shows behavior similar to that of classic PTC with frequent lymph node metastasis. Given the indolent behavior associated with non-invasive encapsulated FVPTC, a proposal for a new term without the use of the word “carcinoma” is currently deliberated and set for publication. The reclassification of non-invasive encapsulated FVPTC into a “non-malignant” category will alter the malignancy risk for each cytopathology diagnostic category and may potentially affect the behavior of cytopathologists regarding the incidence and distribution of each cytopathologic diagnosis. The potential decrease in the positive predictive value for the malignant diagnosis to ~94% may become a concern for cytopathology practices. Furthermore, the reclassification of non-invasive encapsulated FVPTC to a non-malignant category will likely decrease the positive predictive value of certain molecular tests associated with follicular-patterned neoplasms (e.g., RAS, PAX8-PPARγ, BRAF K601E, EIF1AX, GNAS, PTEN, TSHR). Nonetheless, the integration of ultrasound imaging, cytopathologic, and molecular testing results would most likely become valuable in predicting neoplasms such as encapsulated FVPTC (non-invasive encapsulated FVPTC or invasive encapsulated FVPTC). Although resection is the current standard of care for encapsulated FVPTCs, a non-surgical approach may be investigated in the future.

Keywords: Thyroid, indeterminate, molecular, reclassification, follicular variant papillary carcinoma

Introduction – the thyroid cancer dilemma

In counseling a patient with a thyroid nodule, an endocrinologist may state, “I have good news and bad news for you. The bad news is that you have thyroid cancer and need surgery. The good news is that you probably will not die from your thyroid disease.” With this approach and by increasing the sensitivity for detection and treatment of thyroid nodules, mortality from thyroid cancer has remained very low. Most resected thyroid carcinomas are Stage I or II and the five-year relative survival for these low-stage thyroid carcinomas is greater than 98% (1). Superficially, this may appear to be a victory on the “War on Cancer.” However, there are several underlying issues that demonstrate the complexities of this matter.

First, the reported “incidence” of thyroid cancer is increasing. While some major cancers such as colorectal carcinoma are declining in incidence, thyroid cancer, currently the 8th most common cancer, is projected to become the 4th leading cancer by the year 2030 (2, 3). In the state of Pennsylvania (where the author resides), the incidence has been reported to be increasing at a faster rate than the rest of the United States (4). Is this cause for alarm? Investigation into this issue reveals that the causes for this “growth in incidence” are multifactorial and include heightened clinical awareness, improved radiologic detection, increased histopathologic sensitivity for the diagnosis of carcinoma, and the changing biology of thyroid neoplasms. Also, during this time of increased reported incidence, the mortality from thyroid cancer has remained flat (5). The implication is that most of the newly discovered cancers represent indolent disease. In a recent study from the United States, remarkable changes in the distribution of cancer types and the associated mutations were found over the recent four decades (6). Specifically, an increase in the proportion of follicular variant papillary thyroid carcinoma (FVPTC) and a decrease in classic papillary thyroid carcinoma (PTC) were noted. Along with these changes, the proportion of neoplasms with RAS mutations increased from 3% to 25%, while the proportion of RET/PTC rearrangements decreased from 11% to 2%. The proportion of neoplasms with BRAF mutation was stable (~46%). These results suggest the decreased effect of radiation (which often is associated with RET/PTC rearrangement) and involvement of other etiologic factors that are yet to be discovered.
Table 1. Subtypes of papillary thyroid carcinoma (PTC)

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Characteristic Features</th>
</tr>
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<tbody>
<tr>
<td>Classic PTC</td>
<td>Papillary architecture. Frequently associated with \textit{BRAF} V600E mutation or \textit{RET/PTC} gene rearrangement.</td>
</tr>
<tr>
<td>Follicular Variant PTC</td>
<td>Follicular-patterned architecture. Frequently associated with \textit{RAS} mutation or \textit{PAX8-PPARY} gene rearrangement.</td>
</tr>
<tr>
<td>Oncocytic Variant PTC</td>
<td>Oncocytic neoplastic cells with “papillary” nuclear features arranged in papillary or follicular pattern.</td>
</tr>
<tr>
<td>Tall Cell Variant PTC</td>
<td>“Tall” neoplastic cells with characteristic “papillary” nuclear features arranged in papillary, trabecular or cord-like pattern.</td>
</tr>
<tr>
<td>Clear Cell Variant PTC</td>
<td>Clear neoplastic cells in papillary or follicular pattern.</td>
</tr>
<tr>
<td>Diffuse Sclerosing Variant PTC</td>
<td>Diffuse involvement of thyroid gland usually without forming a discrete mass. Often associated with psammoma bodies, lymphocytic infiltrates and fibrosis.</td>
</tr>
<tr>
<td>Columnar Cell Variant PTC</td>
<td>Columnar neoplastic cells with supranuclear and subnuclear vacuoles. Characteristic nuclear features of PTC may be found only focally.</td>
</tr>
<tr>
<td>Solid Variant PTC</td>
<td>Solid sheets of tumor cells often associated with extra-thyroidal extension and vascular invasion in children who had radiation exposure.</td>
</tr>
<tr>
<td>Cribriform Morular Variant PTC</td>
<td>Cribriform architecture of neoplastic cells along with solid and/or papillary features. Spindle cells and squamous morules may be present. Associated with Familial Adenomatous Polyposis or Gardner syndrome.</td>
</tr>
<tr>
<td>Warthin-like Variant PTC</td>
<td>Oncocytic neoplastic cells with abundance of infiltrating lymphocytes, often associated with lymphocytic thyroiditis.</td>
</tr>
<tr>
<td>Hobnail Variant PTC</td>
<td>Aggressive variant of PTC showing micropapillary architecture with apocrine features of neoplastic cells and loss of nuclear polarity.</td>
</tr>
<tr>
<td>Papillary Microcarcinoma</td>
<td>Size $\leq 1.0$ cm. By definition, incidental finding. Histology may show various patterns.</td>
</tr>
<tr>
<td>Others</td>
<td>PTC may be seen in combination with other patterns or types of carcinoma (e.g., PTC with fasciitis-like stroma, PTC with focal insular component, combined PTC and medullary carcinoma).</td>
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</table>

Second, a group of oncologists has attributed the “increased incidence” mainly to increased detection and overdiagnosis. They advocate change to the cancer terminology for indolent neoplasms, reserving the term “cancer” or “carcinoma” for those tumors that would have a reasonable likelihood of lethal behavior if untreated (7, 8). Such alteration may influence the management of thyroid nodules since some thyroid surgeries are performed as “diagnostic” resections for pre-operatively indeterminate thyroid fine needle aspiration (FNA) diagnoses. Removing the term “carcinoma” from the lowest grade thyroid carcinomas may ease the urge to refer a patient to surgery for a relatively low-risk indeterminate FNA diagnosis and reduce overtreatment. In other words, the change is intended to prevent taking benign nodules and indolent thyroid neoplasms as “casualties” on the War on Cancer. Patients with low risk evaluation results who do not opt for surgery may be followed medically and the decision for surgery may be made when significant alterations are noted clinically.

Third, while the majority of thyroid neoplasms are indolent, there are some which are potentially aggressive and others that behave very aggressively. Stage III and IV thyroid carcinomas (which comprise approximately 18% of all differentiated thyroid carcinomas) are potentially aggressive and demonstrate a relative 5-year survival of 71% to 93% and 10-year survival of 37% to 77%, depending on the histologic type (9, 10). Even some Stage I and II thyroid carcinomas are potentially aggressive when followed over 10 years. Although rare, undifferentiated (anaplastic) carcinoma is highly aggressive and has a median survival of 9 weeks and 5-year survival of 7% (9). Overall, there are three major clinico-pathologic groups of thyroid neoplasms: 1. Indolent (e.g. adenomas, noninvasive encapsulated FVPTCs, most papillary microcarcinomas); 2. Potentially Aggressive (e.g. some Stage I and II and most Stage III and IV carcinomas); and 3. Highly Aggressive (undifferentiated carcinoma). Therefore, another reason for the widening gap between the increasing incidence and flat mortality line is that “early” surgical resection of potentially aggressive cancers may have prevented disease progression in some cases.

The dilemma in thyroid nodule diagnostics and management is the prevention of morbidity and mortality from potentially aggressive and highly aggressive cancers while minimizing the surgical overtreatment. The root cause regarding this problem is the cytologic and histologic overlap in the morphology of benign lesions and potentially aggressive neoplasms. In realizing this goal, where should the threshold for surgery be set? Should all thyroid nodules that have the possibility of being called “carcinoma” be resected? Development in the recent decades has provided some answers that are calling for change.
Papillary thyroid carcinoma and the upcoming changes for low grade neoplasms

While the major types of primary thyroid cancers have been well established for decades, the incidence of these entities has varied depending on the geographic region and has changed over the recent decades. According to the 2004 World Health Organization (WHO) Classification of Tumors, the estimated incidences for the thyroid cancer types are as follows: PTC, 70-75%; follicular carcinoma (FC), 10-15%; medullary thyroid carcinoma, 5-10%; poorly differentiated carcinoma, 4-7%; and undifferentiated carcinoma, <5% (10). Over the recent years in North America, PTC has increased further in incidence largely in part due to the rise of FVPTCs while FC has become a rare entity. At our institution, PTC comprised 97% and FC only 0.7% of 1,510 resected thyroid cancers over the last several years (2007-2013) (11). It would not be an exaggeration to state that the overall rise in incidence of thyroid cancer has been due mainly to the rise in incidence of PTC (particularly, FVPTC). To better understand the shift in thyroid cancer incidence, let us review the subtypes of PTC and related entities (Table 1). The diagnosis of PTC originally was based histologically on papillary architectural features. Over the recent decades, the definition and criteria have shifted to emphasize the nuclear features (nuclear enlargement, clearing, grooves, and pseudo-inclusions) and this change has influenced cytopathologists, surgical pathologists and managing clinicians. Unfortunately, the nuclear features may not be uniformly developed throughout the entire neoplasm and in some cases, the nuclear features may be quite subtle and subject to interpretation by the observer.

Broadly, PTC may be divided into types with obvious nuclear features (e.g., classic PTC, tall cell variant PTC, diffuse sclerosing variant PTC, hobnail variant PTC, wide invasive (diffuse infiltrative) FVPTC) and those with more subtle nuclear features (e.g., encapsulated or well circumscribed FVPTC). What we know today as FVPTC was first described by Crile and Hazard in 1953 (as “alveolar variant of PTC”) and phrased as “follicular variant of papillary carcinoma” by Lindsay in 1960 (12, 13). Chen and Rosai in 1977 supported the term “Follicular Variant of Thyroid Papillary Carcinoma” and emphasized the presence of “ground-glass” nuclear features in these tumors (14). Gradually, during the latter part of the 20th century, FVPTC gained popularity and the diagnosis of PTC became an entity that was primarily based on nuclear features. FVPTC primarily demonstrated follicular-type growth pattern while the typical papillary architecture may be observed focally within a tumor. As stated earlier, the rise in incidence in PTC has been attributed mostly to FVPTC and some of these cases resulted from the shift in diagnostic trend that favored the FVPTC diagnosis over FC and follicular adenoma (FA). In addition, there may have been other de novo cases that developed as a result of an increased incidence in RAS mutations in some regions (Figure 1) (6).

Early in the 21st century, studies demonstrated that FVPTC may be divided further into a) encapsulated and well circumscribed and b) infiltrative/diffuse types (15). The histopathologic diagnosis of infiltrative/diffuse FVPTC is relatively straightforward and these tumors behave more similarly to classic PTC with frequent lymph node metastasis. For encapsulated and well-circumscribed tumors, the decision by the surgical pathologist to make the diagnosis of FVPTC is based primarily on nuclear features. If capsular or vascular invasion is present, the differential diagnosis involves FC. In cases lacking capsular invasion, the differential diagnosis includes FA and other benign entities. For borderline cases, the determination is in part related to the subjective willingness of the surgical pathologist to take the risk of making a false negative diagnosis since rare cases of distant metastasis from encapsulated FVPTC have been reported (16, 17). Given the concern for these rare metastatic events and potential medico-legal issues, the threshold for making the diagnosis of FVPTC (even when encapsulated) has become quite low in the United States (18). However, this topic has engendered much controversy and marked observer variability has been noted among thyroid pathology experts in the United States and other countries (19-21). For some controversial thyroid nodules, the expert diagnoses has ranged from FVPTC to FC to FA. While the characteristic nuclear features of PTC (i.e., intranuclear pseudo-inclusions, nuclear grooves, nuclear overlapping, ground-glass chromatin and nuclear clearing, and nuclear membrane irregularity) are well accepted by most thyroid pathologists (20), the degree to which these features are required for establishing the FVPTC diagnosis varies from individual to individual. In particular, the interpretation of ground-glass chromatin and nuclear clearing is perhaps most controversial. Different approaches to encapsulated and well-circumscribed

![Changes in Proportions of Follicular Patterned Thyroid Neoplasms](image)

Fig. 1. Over the recent decades, the proportion of follicular adenoma and follicular carcinoma has decreased, while the proportion of FVPTC has increased. FA, follicular adenoma; FC, follicular carcinoma; FVPTC, follicular variant papillary carcinoma; non-inv, non-invasive; inv, invasive; enc, encapsulated.

![Non-invasive Encapsulated FVPTC](image)

![Invasive Encapsulated FVPTC](image)

![Infiltrative FVPTC](image)

Fig. 2. Diagrammatic illustrations of follicular variant papillary thyroid carcinoma subtypes. PTC, papillary thyroid carcinoma; FVPTC, follicular variant papillary carcinoma; Ca, carcinoma; rec, recurrence; met, metastasis.
tumors have been recognized in Japan where a more conservative approach is advocated. Studies from Japan propose that encapsulated follicular-patterned tumors with equivocal nuclear features of PTC (nuclear grooves, ground-glass chromatin and nuclear clearing without intranuclear pseudoinclusions) are best classified as “well-differentiated tumor of uncertain malignant potential” (WDT-UMP) since follow-up studies of these cases show no evidence of recurrence (22-24). However, in the United States, these tumors are often diagnosed as encapsulated FVPTC because of the concern regarding metastatic disease. Whether these differences in approaches are based on pathology practice patterns (e.g., microscopic interpretation or how extensively the capsule is examined histologically for transcapsular or vascular invasion) and/or underlying endemic differences in the biology of these follicular-patterned tumors (from different regions of the world) require further investigation. Nonetheless, recent interest in the follicular-patterned tumors has led to further subclassification of FVPTC into the following: 1) non-invasive encapsulated (including partially encapsulated and well-circumscribed types) FVPTC; 2) invasive encapsulated FVPTC; and 3) infiltrative FVPTC (Figure 2) (25). Furthermore, molecular and clinicopathologic studies on these tumors have shown that non-invasive encapsulated FVPTC and invasive encapsulated FVPTC show profiles more similar to that of FA and FC (RAS mutations and PAX8-PPARγ translocations) in contrast to that of classic PTC (BRAF V600E mutation and RET/PTC translocations) (17, 26-29). In particular, non-invasive encapsulated FVPTC was recognized as an indolent tumor with behavior similar to that of FA. By comparison, Ganly, et al. recently demonstrated that invasive encapsulated FVPTCs (which show nuclear features similar to that of non-invasive encapsulated FVPTCs) were significantly larger (46% greater than 4 cm) and had higher rates of adverse outcome of recurrence (16%) and mortality (12%) than their noninvasive counterpart (0% recurrence and mortality) (29). Therefore, invasive encapsulated FVPTC would be considered a potentially aggressive PTC for which the clinicians would want to prevent morbidity and mortality. Given the recent trend to remove the word “carcinoma” from very indolent tumors, an international task force of expert thyroid pathologists, endocrinologists, and surgeons convened in an adjunct session at the United States and Canadian Academy of Pathology Meeting in Boston, Massachusetts, in March 2015 to discuss and establish a new term for non-invasive encapsulated FVPTC ( provisionally stated as Non-invasive Follicular Thyroid Neoplasm with papillary-like nuclear features or NIFT). The proceedings from this meeting and the official term for the non-invasive encapsulated FVPTC category will be set in a forthcoming publication.

How will the new classification influence the thyroid cytology practice?

The reclassification of non-invasive encapsulated FVPTC into a “non-malignant” category can have at least a twofold effect on cytology practice. First, the reclassification will alter the malignancy risk for each cytology diagnostic category (e.g., the Bethesda System for Reporting Thyroid Cytopathology – BSRTC) (30) since what is currently considered the lowest grade papillary carcinoma will no longer be classified as a malignant neoplasm. Although the new terminology has not yet been established, Strickland et al. recently investigated the impact of the reclassification of noninvasive FVPTC (corresponding to non-invasive encapsulated FVPTC) on the malignancy rate for each thyroid FNA diagnostic category (31). They showed that the malignancy risk for each BSRTC category will decrease (non-diagnostic (ND) - 18.9% to 17.0%, benign/negative (BN) - 13.2% to 5.4%, atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) - 39.2% to 21.6%, follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) - 45.5% to 37.5%, suspicious for malignancy (SM) - 87.2% to 45.7%, and positive/malignant (PM) - 98.7% to 93.6%). While the most striking decrease is seen in the SM category (87.2% to 45.7%), the drop in malignancy risk for the PM category indicates a potential increase in the false positive rate from 1.3% to 6.4%, if the cytologic threshold for malignancy is maintained. This study also detailed the distribution of the pre-surgical BSRTC categories for the subsequently resected non-invasive encapsulated FVPTC cases: ND - 4%; BN - 13% AUS/FLUS - 18%, FN/SFN - 10%, SM - 49%, and PM - 7% (31, 32). These results (especially the low rate of pre-surgical PM diagnosis) are not unexpected since the resected non-invasive encapsulated FVPTC often show equivocal and subtle nuclear features of PTC that may not be fully and uniformly developed throughout the neoplasm. Although the institutions of this study primarily uses ThinPrep® (Hologic, Inc, Marlborough, MA) liquid based monolayer technology only for cytology processing (33), Ustun et al. (who use direct smears and ThinPrep® processing) have also observed this phenomenon (34). Another observation - the low incidence of FN/SFN diagnosis (10%) is somewhat surprising since non-invasive encapsulated FVPTC is a follicular-patterned neoplasm. However, this finding may be attributed to the fact that FVPTCs often show a partial macrofollicular pattern and the corresponding cytology specimens may demonstrate “colloid-rich” features (35). If the more typical microfollicular areas of FN/SFN are not sampled, such cases of non-invasive encapsulated FVPTC may be classified as AUS/FLUS, SM, or even BN. For these cases, the cytologic diagnosis primarily is based on the nuclear (not architectural) features. Approximately one-half (49%) of the non-invasive encapsulated FVPTC cases had a pre-surgical FNA diagnosis of SM, indicating that many of these FNA cytology cases probably demonstrated equivocal and subtle nuclear features of PTC (ground-glass chromatin and nuclear clearing) whether in a flat sheet-like pattern or a microfollicular pattern. Whether the equivocal and subtle nuclear features of PTC (ground-glass chromatin and nuclear clearing) will be interpreted in the same manner after the reclassification leads to the next discussion point.

The second area potentially affected by the reclassification involves the potential change in behavior of cytopathologists regarding the incidence and distribution of each cytologic diagnostic category. By nature, the cytologic diagnostic categories of FVPTC is challenging - FVPTC may or may not show microfollicles and the nuclear features (ground-glass chromatin and clearing) may be subtle and focal (34). Given the data compiled by Strickland et al., the subtle nuclear cytologic features (ground-glass chromatin and clearing) will become less specific for PTC and contribute to a higher false positive FNA cytology diagnosis (31). Once this loss in specificity is recognized by cytopathologists, cases that were previously diagnosed as PM (based on ground-glass chromatin and nuclear clearing features alone) may become downgraded to SM or AUS/FLUS. The PM diagnosis may become reserved for FNA cytology cases that demonstrate the robust cytologic features of PTC (nuclear grooves and pseudoinclusions). The extent to which the reclassification will influence the cytopathologist’s behavior will depend on a number of factors that have been in effect at individual institutions. Some surgical pathology practices already may have been calling non-invasive encapsulated FVPTC cases “benign.” For these, the reclassification will have little influence on cytology. Also, cytology practices that had already set a high
threshold for establishing a PM cytology diagnosis (e.g., requiring nuclear grooves and pseudoinclusions) may notice little change. Other practices may view FNA thyroid cytology as “screening” tools and not be too bothered by false positive diagnoses. On the other hand, for those practices expecting FNA thyroid cytology diagnoses to be as accurate as surgical pathology biopsy diagnoses, the risk of malignancy of ~94% for the PM diagnosis may be too low for comfort and some change in practice patterns may be implemented. At the other end of the diagnostic spectrum, the risk of malignancy for the BN diagnosis is expected to decrease (from 13.2% to 5.4% according to Strickland et al.), resulting in a lower false negative rate. While this may appear as a welcoming change, we do not know if the reclassification will encourage more BN cytology diagnoses and lead to more false negative diagnosis of potentially aggressive (i.e., invasive) PTCs. After all, the distinction between non-invasive encapsulated FVPTC and invasive encapsulated FVPTC is based on invasion alone; the neoplastic cells within the capsule would be indistinguishable from those in the noninvasive counterpart. In this matter, this situation is analogous to the distinction between FA and FC. Much remains to be proven following the reclassification.

The impact of molecular testing on follicular patterned lesions

The recently compiled Cancer Genome Atlas (TCGA) project expanded the proportion of PTC with known oncogenic mutations to approximately 97% (36). One of the key findings of this study was the fundamental molecular difference between the BRAF V600E-like and RAS-like PTCs. BRAF V600E-like PTCs show activation of the MAP-kinase pathway and high output of the ERK transcriptional program. In contrast, RAS-like PTCs activate both the MAP-kinase and the PI3K/AKT pathways through different mechanisms. Since differentiated PTCs, often show mutations that are mutually exclusive of each other, the identification of specific mutations is helpful in predicting the histologic classification and behavior of tumors. In this regard, thyroid carcinomas with BRAF V600E and RET/PTC genetic alterations have a very high positive predictive value (~99%), are associated with classic PTC or tall cell variant PTC and more commonly present as stage III or IV. In contrast, thyroid carcinomas with RAS, PAX8-PPARγ, and BRAF K601E mutations are associated with follicular-patterned neoplasms such as FVPTC or FC that often present as stage I or II (11, 37). More recently, the next generation sequencing (NGS) assay on cytology samples has expanded the list of mutations associated with follicular-patterned neoplasms (e.g., EIF1AX, GNAS, PTEN, TSHR). However, the association of these less frequent mutations with carcinoma is variable and may not be as strong as that for RAS, PAX8-PPARγ, and BRAF K601E mutations (38-40). Further studies are needed to characterize the nature of the less frequent mutations more precisely.

The reclassification of non-invasive encapsulated FVPTC to a non-malignant category will affect the positive predictive value of mutations associated with follicular-patterned neoplasms (e.g., RAS, PAX8-PPARγ, BRAF K601E, EIF1AX, GNAS, PTEN, TSHR) and is expected to decrease the value below the current average of 89%. Recently, Medici et al. performed a follow-up study (both clinical and surgical) on RAS-positive thyroid nodules using miRInform Thyroid® molecular test (Asuragen Inc. Austin, TX, USA - now Interpace Diagnostics, Parsippany, NJ) (41). Of the 362 thyroid nodules evaluated, 17 were found to be RAS-positive. Ten of these nodules were resected and eight showed FVPTC (80%). Seven (87.5%) of these would qualify as non-invasive encapsulated FVPTC and one showed potential capsular invasion. For the remaining nodules that were not resected, 6 of them had a “benign” cytology diagnosis and one AUS/FLUS case that showed a “benign” result by Afirma® gene expression classifier (Veracyte Corp, South San Francisco, CA). Therefore, if these clinically “benign” cases were included in the total, the positive predictive value for the RAS-positive cases would be 47%. However, the true histologic classification of the unresected nodules cannot be determined since the capsule had not been histologically evaluated - although a substantial portion would be expected to represent non-invasive encapsulated FVPTC.

Another valuable aspect of multi-gene molecular panel tests (e.g., ThyroSeq v2 NGS, Afirma® gene expression classifier) lies in the accuracy of a negative test result. Previous studies on the malignancy risk of negative ThyroSeq v2 NGS cases with FN/SFN and AUS/FLUS thyroid cytology diagnoses showed carcinoma outcome in 4% and 3% of cases, respectively (39, 40). Afirma® gene expression classifier studies claimed comparable results for their molecular negative FN/SFN (6% malignancy risk) and AUS/FLUS (5% malignancy risk) cases (42). Needless to say, these values should shift downward with reclassification of non-invasive encapsulated FVPTC to a non-malignant category.

Integrating the clinical, cytologic, and molecular diagnostic modalities for improved management

With technological advancements, the triad of ultrasound imaging, cytopathologic evaluation and molecular testing may become the new “triple test” in thyroid nodule assessment. By utilizing ultrasonography, nodular characteristics such as echogeneity, shape (tall versus round), borders (infiltrative versus non-infiltrative), and presence or absence of calcifications are evaluated. Although ultrasonography alone lacks high specificity and ultimate precision in classifying a nodule, it provides valuable “gross” diagnostic information that separates radiologically infiltrative from non-infiltrative nodules and may predict follicular-patterned neoplasms such as FVPTC (43-45). Combined with cytologic (e.g., AUS/FLUS and FN/SFN) and molecular (e.g., RAS mutation) information, cases that will likely result in encapsulated FVPTC (non-invasive encapsulated FVPTC or invasive encapsulated FVPTC) may be predicted. The next challenge would be determining the most appropriate management for such cases - whether these nodules should be resected (since capsular invasion cannot be evaluated without resection) or if they may be observed and followed over time. Long term follow-up studies on unresected PTCs are sparse in the literature. Prospective active surveillance studies by Ito et al. on low risk papillary thyroid microcarcinomas showed that although a small percent of these patients developed lymph node metastasis after 5 years (1.4%) and 10 years (3.4%), none of the patients died from PTC (46, 47). On the other hand, rare reports of fatal papillary thyroid microcarcinomas without BRAF V600E, NRAS, HRAS, or KRAS mutation raise concern to those who practice in areas where medicolegal issues need to be taken in consideration (48). Perhaps the reclassification of non-invasive encapsulated FVPTC will shift the focus from the subtle nuclear features of PTC to sampling of the capsule and the interpretation of capsular and vascular invasion since the study by Ganly et al. reported 12% mortality in patients with invasive encapsulated FVPTCs (29). The paradigm has shifted; however, the dilemma regarding the optimal setpoint for diagnosis and management remains.

Conflicts of Interest: None
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