

## REVIEW

# Thyroid nodule management: clinical, ultrasound and cytopathological parameters for predicting malignancy

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Although fine-needle aspiration cytology is considered to be the reference method for evaluating thyroid nodules, the results are inaccurate in approximately 10-30% of cases. Several studies have attempted to predict the risk of malignancy in thyroid nodules based on age, nodularity, thyrotropin values, thyroid autoimmune disease, hot/cold nodule status, and ultrasound parameters. However, no consensus has been found, and none of these parameters has significantly affected patient management. The management of indeterminate thyroid nodules and re-biopsies of nodules with initially benign cytological results remain important and controversial topics of discussion. The Bethesda cytological system and several studies on the use of molecular markers to predict malignancy from cytological samples of thyroid nodules need further clarification. More in-depth discussions among and continuous education of the specialists involved in treating thyroid disease are necessary to improve the management of these patients. This review aims to examine the clinical, laboratory, ultrasound, and scintigraphic parameters that can be used for thyroid nodule management.

**KEYWORDS:** Thyroid Nodule; Ultrasound; Fine-Needle Aspiration Cytology; Malignancy.

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## THYROID NODULES

Thyroid nodules are one of the most common endocrine diseases in the world. They affect approximately 4 to 7% of the population in iodine-sufficient areas, with a markedly increased incidence in iodine-deficient regions (1). Thyroid nodules are classified as adenomas, carcinomas, or hyperplastic lesions based on their macroscopic and microscopic histological features (1,2).

Adenomas consist of encapsulated lesions derived from the follicular epithelium, and they may be present in isolated, macrofollicular (colloid), microfollicular (fetal), and trabecular/solid (embryonic) forms (2,3). Adenomas may be functioning (autonomous), in which case they are proportionally larger than the rest of the parenchyma and produce excessive thyroid hormones, or non-functioning, in which case hormone levels are unchanged. Autonomous adenomas can occur at any age, but they are rarely toxic in individuals under 60 years of age (4). These nodules are generally considered benign, with rare cases of malignancy (5).

Nodular hyperplastic lesions are characteristically present in multinodular goiter (MNG) and are caused by follicular cell hyperplasia. In some cases, hyperplastic nodules can grow and become autonomous even in the absence of external stimuli (6).

Differentiated thyroid carcinomas (DTCs), which encompass papillary and follicular carcinomas, are relatively uncommon tumors. They are generally associated with a good prognosis, with an estimated incidence of 1 to 10 cases/100,000 people per year. They are the most common endocrine neoplasm in the world, but they represent only 1% of all malignancies (1,7,9). Undifferentiated or anaplastic carcinomas represent approximately 5% of all thyroid carcinomas, and medullary thyroid carcinoma (MTC), which is derived from parafollicular cells, may occur sporadically or familiarly (1,3,5,8).

Due to the increased use of ultrasonography (US) and the increased access to cytology analysis through fine-needle aspiration biopsy (FNAB) guided by US (FNAB-US), the number of small-sized thyroid gland carcinoma diagnoses has increased in Brazil and in many other countries (5-8). Thus, carcinomas smaller than 1 cm in diameter are being detected more frequently. They are usually diagnosed in an unexpected manner ("incidentalomas") by US or histopathological examinations of surgically excised glands in cases with benign presentations, such as airway obstruction, large goiter, and uncontrolled hyperthyroidism (8,9).

Epidemiological studies conducted in iodine-sufficient regions demonstrate a 5 to 10% prevalence of palpable

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thyroid nodules in women and a 1 to 2% prevalence in men (1,2,8-10). US studies have revealed the existence of thyroid nodules in 19 to 67% of normal-risk female and elderly individuals (3,10-14). These findings have been corroborated by autopsy studies (15). The increased nodularity and diameter of the thyroid seems to be inversely proportional to thyroid-stimulating hormone (TSH) levels (16-18). An evolution to hyperthyroidism due to the development of functioning autonomous nodules occurs in approximately 10% of cases over a ten-year period (19-21).

FNAB-US assessment is currently recommended for nodules with a diameter larger than 1 cm and for nodules smaller than 1 cm with suspicious features (hypoechoogenicity, microcalcifications, border irregularity, and Doppler central flow) (8,9,13,22,23) detected by ultrasound. An ultrasound examination is indicated in all cases of suspected nodules, and an initial TSH serum assessment should be performed in addition to scintigraphy when a functional nodule is confirmed (9).

The majority of patients with thyroid nodules can be treated conservatively because 90 to 95% of nodules are non-functional, benign nodules with associated mortality rates of less than 1% (1,8,9,13). In particular, female gender, age between 20 and 45 years, nodules smaller than 2 cm, lack of multicentricity on US, absence of a glandular capsule, and locoregional lymph node outbreaks are considered to be factors related to low malignancy risk (8,9,13,16). Thus, it is important to properly select candidates for surgery based on the suspicion of malignancy (16,21,22).

Various clinical, ultrasound, and cytological parameters, such as age, gender, nodularity, TSH level, thyroid autoimmunity, and ultrasound findings (hypoechoogenicity, microcalcifications, irregular borders, and increased central nodular flow), have been studied to improve diagnostic accuracy and differentiate between benign and malignant nodules (9,13-16,22). The literature indicates higher malignancy rates in individuals below 16 or above 45 years of age (8,13). There is no male or female predominance, even though the incidence of nodules is higher in women (8,9). Some studies have found a higher malignancy rate in patients with a solitary nodule than in patients with multiple lesions (21), although more recent reports have not confirmed this association (8,22). Therefore, the results are conflicting, and the samples studied are often not representative, due to either short follow-up times or low correlations between the factors studied (1,2,8,10,13-16).

Cytopathologically characterizing and differentiating between benign and malignant follicular lesions is practically impossible (24). The identification of genetic or immunohistochemical markers that can distinguish both follicular adenoma from follicular carcinoma and papillary hyperplasia from papillary carcinoma remains a long-standing goal. However, these markers remain inadequate for use in clinical practice (24-27).

This review aims to discuss current thyroid nodule management, diagnosis, and malignancy prediction using ultrasound imaging and clinical and cytopathological data. The following terms were used in a Medline/Pubmed search: thyroid nodule, management of thyroid nodules, thyroid ultrasound, thyroid cytology, and FNAB of thyroid nodules. Approximately 220 articles published in the period from 2000 to June 2011 were analyzed. The consensual results pertinent to the topic were described to both examine

the major diagnostic concerns and to explore more accurate therapeutic approaches for patients with thyroid nodules.

## TSH LEVELS AND AUTOIMMUNE DISEASE IN THYROID NODULES

Previous studies have shown that increased serum TSH levels may be associated with an increased risk of thyroid cancer in patients with nodular goiter (28-37). The risk of thyroid malignancy increases with higher TSH concentrations, even those within the normal range (28-30). Boelaert et al. (30) studied 1500 consecutive patients without thyroid dysfunction and found a higher risk of malignancy (an adjusted odds ratio (AOR) of 2.72) in subjects whose TSH levels ranged from 1.0 to 1.7 mU/L than in those with TSH levels <0.4 mU/L (an AOR of 1.00), with a particularly high incidence found in those with TSH levels >1.8 mU/L (AOR 3.88). Males, younger patients, and patients with solitary nodules were also found to have a higher risk of malignancy (30).

Fiore et al. (37) studied the relationship between TSH serum levels and papillary thyroid carcinoma (PTC) in patients with uni- or multi-nodular goiter who were or were not treated with levothyroxine. The treated patients had lower TSH serum levels and decreased PTC prevalence. The PTC prevalence was lower in the patients with TSH levels <0.4 mU/ml and was greater in those with TSH levels >3.4 mU/ml (1.9% vs. 16.5%), with no influence of age or multiple nodularity.

In contrast, Gerschpacher et al. (35) compared the TSH concentrations of 33 patients with papillary microcarcinoma who underwent total thyroidectomy with those of a control group with carcinomas larger than 1 cm (n=54), and no significant TSH concentration differences were observed ( $1.40 \pm 0.92$  mU/L vs.  $1.43 \pm 1.44$  mU/L). Moreover, these results were not observed in the patients with indeterminate nodules based on cytology (29,31-36).

Retrospective studies have reported a correlation between thyroid malignancy and autoimmune thyroid disease (ATD) (33-39), as well as a higher rate of malignancy in Hashimoto's thyroiditis (HT) nodules (22). In contrast, Anil et al. (38) observed a 1.0% malignancy rate in patients with HT vs. a 2.7% rate in a control group, a difference that was not statistically significant. Mukasa et al. (40) observed a higher malignancy rate in HT patients with nodules larger than 1 cm or with smaller nodules exhibiting suspicious US findings than in patients with Graves' disease (1.77% vs. 0.97%). Adenomatous lesions were also more frequent in the HT group and in patients younger than 40 years of age. Thus, the most recent reports recommend measuring anti-TPO and anti-thyroglobulin (anti-Tg) antibodies during initial thyroid nodule investigations in which an elevated TSH level (over the normal range) is found (8,9,22).

## SCINTIGRAPHY AND THYROID NODULES

Scintigraphy and thyroid uptake have been utilized for over 60 years. They are valuable procedures for investigating a number of thyroid dysfunctions, such as destructive thyroiditis, ectopic thyroid, and hyperfunctioning nodules. However, they have limited diagnostic value for iso- or hypo-functioning thyroid nodules (8-10,41,42). Scintigraphy (and thyroid uptake with radioactive iodine or perthecnetate- $Tc^{99m}$  when TSH is subnormal) is recommended for

evaluating functional nodules (9,22). Hyperfunctioning nodules are almost always benign, while non-functioning nodules carry estimated malignancy risk rates of 10 to 20% (8,9,22). Additionally, scintigraphy is indicated for determining the functional status of nodules with indeterminate cytology; the goal is to detect hot nodules that are probable follicular adenomas and to differentiate between the nodules in multinodular goiters (43).

Due to improved nuclear imaging methods, studies using dynamic nuclear magnetic resonance (NMR) imaging have become increasingly frequent. Gupta et al. (44) recently studied the impact of NMR spectroscopy techniques on the detection of thyroid follicular neoplasms. Choline peaks were observed in eight of the analyzed cases of follicular carcinoma, with a sensitivity of 100% and a specificity of 94%. Due to the limited follow-up duration and small sample size, the method still needs validation in larger studies. In contrast, Kim et al. (45) did not find FDG-PET to be satisfactory for defining malignancy in the thyroid nodules of 50 patients; therefore, it is still considered to be a low-efficacy method.

**ULTRASOUND OF THYROID NODULES**

Cervical ultrasound is the method of choice for studying thyroid nodules, and it enables the evaluation of the size, location, and characteristics suggestive of malignancies (Figure 1) (8,9,14,22,46-48).

According to Leenhardt et al. (49), hypoechogenicity has a moderate positive predictive value (50 to 63%) for malignancy in thyroid nodules, with high sensitivity (75%) and specificity (61 to 83%) for US examination. Li et al. (50) reviewed the US features of 115 nodules in 104 patients with PTC and found that microcalcifications, central flow, and irregular borders were directly associated with malignant thyroid nodules. Gonzalez-Gonzales (51) studied the US characteristics of 341 thyroid nodules and found that microcalcifications were the only variable that was significantly associated with malignancy.

Moon et al. (32) analyzed 1083 thyroid nodules and found that central flow is the most common distinction between benign and malignant nodules. Of the 1083 nodules studied,

814 were benign and 269 were malignant. Intranodular vascularity was frequently observed in the benign nodules, and vascularity was more typically absent in the malignant nodules. These findings corroborate the results of Cantisani et al. (34), who used Doppler to analyze vascularity in US readings from 1090 patients and concluded that flow pattern should not be used as the sole predictor of malignancy or other thyroid nodule characteristics; therefore, FNAB remains mandatory.

Baier et al. (52) evaluated the US data and the clinical and laboratory characteristics of 944 patients with thyroid nodules and noted an association between malignant solid nodules and patient age younger than 45 years. In contrast, Choi et al. (53) found no association between age and malignancy in nodules with indeterminate cytology. The authors studied the cases of 165 patients who had been diagnosed with "follicular tumors" and found no significant associations between malignancy and gender, age ( $\geq 45$  years), diameter, or US characteristics, although there was a significant association with central flow by Doppler study.

According to the literature, the malignancy rate in thyroid nodules that are 4 cm or larger, with indeterminate cytology, varies from 10 to 30% (22,40,42,49,50). Rosário et al. (45) found malignancies in 23.5% of the cases with indeterminate cytology. They found suspicious characteristics in the ultrasounds of 76% of these nodules, compared with 6.5% of the nodules with no suspicious aspects. In a recent analysis, Kihara et al. (54) found no association between nodule size or thyroglobulin level and malignancy risk in 137 surgically treated patients. However, they observed that malignancy was directly associated with suspicious US findings. These findings were similar to those of Maia et al. (56), who assessed the correlations among the cytological variables of the Bethesda system and the clinical, ultrasound, and scintigraphic data from patients with thyroid nodules with indeterminate cytology. Malignancy was found in 68.4% of the nodules with suspicious US characteristics *vs.* 14.8% of those with normal US findings. After the multivariate analysis, border irregularity as observed by US and Bethesda IV category were able to accurately predict malignancy in 76.9% of the thyroid nodules with indeterminate cytology.

Ultrasound Parameters of Malignancy in Thyroid Nodules	
Benign	Malignant
Iso- or hyperechoic	Hypoechoic
Macro calcifications	Microcalcifications
Regular border	Border irregularity
No infiltrative margins	Infiltrative margins
Absent of abnormal cervical lymph nodes	Abnormal cervical lymph nodes
Periphery nodular vascularity	Increased intranodular vascularity

Figure 1 - Ultrasound parameters suggestive of malignancy in thyroid nodules. Adapted from Lew et al. (14).

Stojadinovic et al. (55) studied 216 patients with thyroid nodules who were examined by US and electrical impedance (EIS) scanning prior to FNAB and thyroidectomy. A Bayesian network model successfully predicted malignancy based on the EIS technique. The model's positive and negative predictive values were 83 and 79%, respectively. These studies require the use of this technique on a large scale with elaborate protocols and long-term follow-up to confirm their effectiveness and practicality.

Combinations of ultrasound characteristics and clinical, laboratory, and cytological markers have frequently been examined in studies that aim to establish prediction models for thyroid malignancies (43,46,47,52-55,57-59).

According to the UICC/AJCC, a classification system based on the pTNM parameters and age at diagnosis should be used to categorize the severity of all types of tumors, including thyroid cancer (60,61). Age is one of the criteria in this system, which uses 45 years of age as the cutoff point. This cut-off point was corroborated by Banks et al. (36) and Baier et al. (52).

Although the rate of thyroid nodules is 5 to 11 times higher in females, the annual incidence of thyroid cancer in the United States is approximately 1.2 to 2.6 per 100,000 males and 2.0 to 3.8 per 100,000 females (7,8,10,52,62,64). While some authors believe that males have a 2- to 3-fold greater risk of thyroid nodule malignancy, caution should be exercised in the interpretation of this result (60,65) because other studies have not demonstrated such a difference (56,66,67). Several studies have shown the importance of age and male gender as prognostic markers for thyroid cancer, regardless of the ultrasound characteristics (9,62,63,68,69).

Alves et al. (70) studied clinical, scintigraphic, ultrasound, and cytological predictors and observed that aspiration cytology yielded better results (sensitivity of 94% and specificity of 97%) than scintigraphy (sensitivity of 89% and specificity of 21%) or US (sensitivities ranging from 60 to 100% and specificities ranging from 25 to 69%).

Another study has proposed a risk analysis based on patient age (older than 50 years), nodule size (2.5 cm), and cytological criteria (nuclear atypia and indefinite or suspicious cytological results) (36). For nodules with diameters less than 2.5 cm, the risk of malignancy was increased by 53% for each 1-cm decrease beginning at 2.5 cm. For larger nodules, the risk increased by 39% for each 1-cm increase. The patients with cytology results suspicious for papillary thyroid carcinoma had the greatest risk of malignancy.

Maia et al. (71) evaluated the risk factors for malignancy in 143 patients with thyroid nodules. The FNAB sensitivity and specificity for malignancy were 82.8 and 97.7%, respectively. The age at diagnosis was an independent risk factor for malignancy, with a cutoff point of 38.5 years. The multivariate model showed that age >39 years, nodule size  $\geq 2$  cm, microcalcifications, and border irregularity based on ultrasound study were predictive factors for malignancy, with a combined accuracy of 81.7%.

## FINE-NEEDLE ASPIRATION CYTOLOGY

FNAB still remains the most important method for detecting malignancy in the management and monitoring of thyroid nodules. It has a high sensitivity (65 to 98%) and specificity (72 to 100%) (8,54,56,72), and it has a false-positive rate for cancer detection of 0 to 7% and a false-negative rate of 1 to 11% (8,54).

Physician experience is quite important for performing this procedure, and US-guided FNAB is preferable. Similarly, pathologist experience in interpreting the aspirated material can guide the therapeutic approach. The procedure is relatively simple, quick, safe, low-cost, and devoid of significant complications (8,9,22,40).

Choi et al. (73) found that 16.1% of 3,767 FNAB-US samples were inadequate, largely due to the lack of physician experience, a predominance of cystic lesions, and the presence of macrocalcifications. Additionally, Akgül et al. (74) found no relationship between malignancy and nodule diameter or clinical (age, gender, and functional gland status) and ultrasound aspects in cases with inadequate cytology results. The authors found a 12.6% rate of malignancy in nodules with unsatisfactory cytological results.

Regarding the cytological variables, an indeterminate or "follicular tumor" diagnosis was a significant problem when attempting to identify malignancies. The authors (9) defined four possible cytopathological results: benign, malignant, suspicious for malignancy (follicular neoplasm or Hürthle cell carcinoma), or non-diagnostic. Thus, they estimated that samples with indeterminate results ("follicular tumor") represented approximately 15 to 30% (8,9,22) of their cases. Given that 70 to 80% of the "undetermined" lesions were eventually classified as benign in the final histological analysis (8,16,22,23,43,46,47), surgery recommendations in these cases are problematic (8,23,27,43,46,47).

The Bethesda classification system was created to guide cytopathological diagnoses and to help identify important correlations with malignancy in the final histological study. It consists of a six-category classification system associated with increased risk of malignancy and is based on a cytohistological analysis of 3207 FNAB samples from 2468 patients (Figure 2) (75). This classification system ensures the uniformity of information shared among pathologists, clinicians, and surgeons, and it provides better correlations between malignancy and cytological results, thus enabling more appropriate management.

Given these objectives, several studies have been conducted regarding the cytological parameters that determine malignancy. According to Kelman et al. and Goldstein et al., the presence of cellular atypia in indeterminate cytology nodules indicates a greater likelihood of malignancy (76-78). Lubitz et al. (79) determined that nine of the 17 cytological characteristics examined in a study of 144 patients were associated with malignancy. In their multivariate analysis, only the presence of vascular transgressions and nuclear cracks were correlated with malignancy in the nodules investigated.

Yehuda et al. (80) studied the predictive value of certain cytological variables, including "atypia", when predicting thyroid nodule malignancies in 111 patients and found a 56% malignancy rate in the final histological analysis. Micro-nucleoli, irregular nuclear contours, and dense chromatin were the most frequent characteristics noted in the malignant tumors, and there was an 83% probability of malignancy when these three characteristics were present. However, cellular atypia was present in 66% of the malignant nodules and in 78% of the benign cases, a difference that was not significant.

Kato et al. (81) studied the specificity of 4 cytological variables indicative of "atypia" for predicting malignancy in

<p align="center"><b>The Bethesda System for Reporting Thyroid Cytopathology: Diagnostic Category and Risk of Malignancy</b></p>	<p align="center"><b>Malignant Risk</b></p>
<p><b>I – NONDIAGNOSTIC or UNSATISFACTORY</b> *cyst fluid only; virtually acellular specimen;</p>	<p align="center"><b>1-4%</b></p>
<p><b>II – BENIGN</b> * Benign follicular nodule: adenomatoid nodule, colloid nodule, etc. • Lymphocytic thyroiditis (Hashimoto); • Granulomatous (subacute) thyroiditis; others.</p>	<p align="center"><b>0-3%</b></p>
<p><b>III – ATYPIA OF UNDETERMINED SIGNIFICANCE or FOLLICULAR LESION OF UNDETERMINED SIGNIFICANCE</b> • NOR BENIGN OR MALIGNANT CRITERIA FOR CATEGORIZATION</p>	<p align="center"><b>5-15%</b></p>
<p><b>IV – FOLLICULAR NEOPLASM or SUSPICIOUS FOR A FOLLICULAR NEOPLASM</b></p>	<p align="center"><b>15-30%</b></p>
<p><b>V – SUSPICIOUS FOR MALIGNANCY</b> • Suspicious for papillary carcinoma; • Suspicious for medullary carcinoma; • Suspicious for metastatic carcinoma; • Suspicious for lymphoma; • Others</p>	<p align="center"><b>60-75%</b></p>
<p><b>VI – MALIGNANT</b> • Papillary thyroid carcinoma • Poorly differentiated carcinoma • Medullary thyroid carcinoma; • Anaplastic carcinoma; • Metastatic carcinoma; • Others</p>	<p align="center"><b>97-99%</b></p>

**Figure 2** - The Bethesda cytological classification system and its correlation with the risk of thyroid nodule malignancy. Adapted from Theoharis et al. (60).

466 surgically treated patients with cytologically indeterminate thyroid nodules. The "atypia" FNAB diagnosis was associated with a 42% risk of malignancy. This risk was 7% when there were no atypical features and 81% when there were four or more. When irregularity and nuclear inclusions were present, there was a 79.3% probability of malignancy and 98% specificity, which is similar to the findings of Yehuda (65). In summary, several cytological, clinical, and laboratory parameters have been studied as predictors of malignancy in thyroid nodules, especially in nodules with indeterminate cytology (Figure 3).

Maia et al. (82) evaluated the correlation between the cytological variables of the Bethesda system and clinical, sonographic, and scintigraphic data on indeterminate thyroid nodules. In a sample of nodules with a 25% malignancy rate, category IV of the Bethesda system was an independent predictor of malignancy. A blind review of the cytology

results by a specialized cytopathologist with experience in thyroid studies resulted in a 10.9% reduction in the cases classified as Bethesda category III or IV, most of which were reclassified as benign cytology (category II); these results were confirmed by the post-surgical treatment. Davidov et al. (83) evaluated the Bethesda classification data of 250 patients who had their FNAB results reviewed by a second pathologist. There was diagnostic agreement between the first analysis and the second opinion in 66% of the cases. The highest concordance rate occurred in the malignant cytology group (categories V and VI), while the rate was only 37% in the indeterminate cytology group (categories III and IV). The second opinion increased the FNAB diagnostic accuracy by 14% and reduced the surgery rate by 25%. Such results demonstrate the importance of cytological review by a pathologist experienced in thyroid surgery recommendations for patients with indeterminate cytology (82,83).

Authors / Year	Subjects (N)	Malignant %	Predictors of Malignancy
Cesorismo et al. (1993)	33	15%	Lymphadenopathy
Tyler et al. (1994)	43	19%	Age (> 50yrs.)
Carpi et al. (1996)	70	16%	Cellular Atypia / diameter > 4cm
Tutle et al. (1998)	103	21%	Solitary nodule / male gender
Kelman et al. (2001)	54	13%	Atypia
Goldstein et al. (2002)	83	11%	Atypia
Sclabas (2003)	73	15%	Diameter > 2cm
Giorgadze et al. (2004)	169	45%	Diameter > 2cm / Age > 40 yrs.
Yehuda et al. (2010)	111	56%	Irregular nucleum; micro-nucleoli
Kihara et al. (2011)	137	24%	Suspect ultrasound

Figure 3 - Malignancy parameters in nodules with indeterminate cytology. Adapted from Banks et al. (50) and Yehuda et al. (65).

**TUMOR MARKERS OF THYROID CYTOLOGY (FNAB): INDICATIONS AND CLINICAL APPLICATIONS**

This discussion refers to the accuracy and specificity of methods and molecular markers of thyroid malignancy, as well as to the appropriate timing of the immunocytochemical analysis of FNAB samples. The appropriate indication of molecular immunocytochemical markers of malignancy increased with the diagnostic pitfalls of Bethesda categories III, IV, and V (20,24,25,84,85).

Galectin 3 (Gal-3) immunodetection is one of the most widely studied markers for malignancy in follicular lesions with indeterminate cytology (20,24,25). Bartolazzi et al. (46) examined Gal-3 expression in 1009 thyroid lesion samples and 226 FNAB cytological results, which showed 98% sensitivity and 99% specificity to discriminate benign and malignant lesions. Pennelli et al. (85) corroborated these results by observing an 80% sensitivity and an 86% specificity in a group of one hundred indeterminate cytological nodules.

The BRAF (V600E) mutation, which is characteristic of PTC, has provided greater diagnostic accuracy for nodules with indeterminate cytology and for nodules that are suspicious for malignancy (20,23,26). While researching the BRAF (V600E) mutation, Kim et al. (47) studied 1074 patients with thyroid nodules and observed an increase in the FNAB sensitivity from 67.5 to 89.6% and an increase in the accuracy from 90.9 to 96.6%. In another analysis, Nikiforov et al. (27) reviewed 470 cytology specimens from 328 patients for BRAF mutations, RAS mutations, RET/PTC markers, and PAX8/PPAR gamma mutations. BRAF mutations were the most common finding, and the presence of three mutations was predictive of a malignancy diagnosis in 97% of the confirmed cases.

Cerruti et al. (86) analyzed four protein markers from cytology material (FNAB) to evaluate thyroid nodules with suspected malignancy. Greater diagnostic accuracy was observed when both proteins derived from chromosome 1 (*chromosome 1 open reading frame 24*, C1orf24) and membrane protein 1 (*integral membrane protein 1*, ITM1) were present. Additionally, the BRAF mutation (V600E) was verified in

48% of the 120 papillary carcinoma cases evaluated and occurred more frequently in the classic PTC cases (66%) than in the follicular PTC variant (21%). Furthermore, there was a strong association between the BRAF (V600E) mutation and extra-thyroid invasion, lymph node metastasis, and recurrence risk, indicating that the mutation is an important prognostic marker for classic PTC.

According to Fadda et al. (87), it is possible to identify two risk categories (high and low) in nodules with indeterminate cytology (follicular neoplasms) based on HBME-1 and Gal-3 expression. Indeterminate cytology was present in 50 of 120 surgically treated cases. In these 50 indeterminate tumors, a positive immunohistochemical panel was observed in 76.9% of the cases with malignant nodules in the final histology, and a negative panel (no positive markers) was observed in almost all (96.8%) of the benign cases. These data were corroborated by Kang et al. (88) in an analysis of the BRAFV600E mutation in (preoperative) FNAB samples from 200 surgically treated thyroid nodules. The mutation was present in 63.3% of the malignant cases with initially indeterminate cytology. Therefore, for nodules with indeterminate cytology (Bethesda categories III and IV), negative tumor markers (HBME-1 and Gal-3) in the FNAB sample suggests conservative management, and a positive immunohistochemical panel suggests surgical treatment.

**FOLLOW-UP OF THYROID NODULES WITH INITIALLY BENIGN CYTOLOGICAL RESULTS**

Thyroid nodules with a benign diagnosis in the initial cytological evaluation have long been thought to require only cervical sonographic assessment for long-term follow up, regardless of the results of the US examination. Despite a false-negative rate that has been classically established at 5% (8,9), several authors have demonstrated the value of repeat FNAB studies for certain thyroid nodules with initially benign cytology (43,60,89-93). There is still controversy over what criteria should be used to select such nodules and over whether systematically repeating FNAB

studies to minimize the number of false-negative results is justified. In solid-nodule cases (including mixed nodules with solid portions) where the growth is less than 20% of the diameter in two dimensions, the appropriate US follow-up interval may be as long as every 3 to 5 years (8,22,30,47).

Thus, some aspects of managing nodules with initially benign cytology deserve further discussion. According to some authors, the risk of malignancy is lower for initially benign thyroid nodules without suspicious US characteristics (0.6%) than in those with US results that suggest malignancy (20.4%) (94). Of 122 surgically treated thyroid nodules, 23 (18.8%) of those with initially benign cytology were found to be malignant after being reaccessed by FNAB at an average interval of 15.5 months. The authors concluded that repeated FNAB studies of initially benign nodules with suspicious US results increases malignancy detection during follow-up.

Kwak et al. (89) reviewed sonographic-cytological correlations in 568 patients to determine whether repeated FNAB studies are indicated for thyroid nodule follow-up. The authors found a high risk of malignancy (92 to 98%) in thyroid nodules that were classified as "malignant" or "suspected for malignancy", regardless of the US findings. For nodules with initially benign cytology, however, suspicious US findings correctly predicted malignancy in more than half of the cases (56.6% vs. 2.9%). Repeated FNAB studies revealed "suspected" or "malignant" cytology in 15 (93.8%) of the 16 thyroid carcinomas that were detected during the follow-up. The authors recommended repeated FNAB studies for nodules with initially benign cytology and suspicious US findings.

Studies on the management of supposedly benign ("Thy 2") thyroid nodules using the "Thy 1-5" cytological classification system suggest performing an additional FNAB after 3 to 6 months for diagnostic confirmation and to reduce the false-negative rate, regardless of the clinical or ultrasound findings (13,23).

Illouz et al. (95) analyzed 119 surgically treated thyroid nodules and found that systematically repeating FNAB studies detected 22.7% of the malignant nodules that were undiagnosed in the initial cytology. The authors recommend at least three FNAB studies to reduce the false-negative rate and accurately diagnose malignancy. A retrospective analysis of more than ten thousand FNAB studies demonstrated that the procedure increased the diagnostic accuracy by 8% (from 90 to 98%) when it was sequentially performed (96). The use of repeated biopsies for initially benign nodules reduced the FN misdiagnosis rate from 5.2% to less than 1.3%.

Orlandi et al. (92) studied 799 sequential, annual FNAB-US studies with favorable results. The studies were performed on 302 patients over 2 to 12 years of follow-up. The authors concluded that FNAB monitoring could be discontinued after at least three benign cytology assessments when clinical suspicion was absent.

Flanagan et al. (90) observed that repeating FNAB up to three times increased the sensitivity from 81.7 to 90.4% and reduced the FN rate by 6.7%. Sensitivity did not increase between the third and the fifth procedures in this study, suggesting that up to three systematic FNAB studies are sufficient for making clinical or surgical decisions about suspected malignancies. Similarly, Maia et al. (97) found a malignancy prevalence of 28.5% in nodules with initially

benign cytology that underwent repeated FNAB studies, which raised the malignancy diagnosis rate by 7%. Of these cases, 82.1% were identified by the third FNAB-US (there was a 13-month interval between the first and third procedures). The ultrasound analysis demonstrated that features suggestive of malignancy (microcalcifications, border irregularities, central flow, and hypoechogenicity features) were significantly more common in the malignant nodules group. Kwak et al. (94) found that 25% of nodules with initially benign cytology exhibited significant growth when examined in repeated FNAB studies. However, this group demonstrated an average malignancy rate of 1% compared with a rate of 20% obtained by ultrasound detection of malignant parameters.

Other authors have contested this position and recommended against repeated FNAB studies due to their high specificity and to the low cost-effectiveness of repeating the procedure in large numbers of nodules that have been diagnosed as benign (98-100). Aguilar et al. (101) found no changes from the initial diagnosis in 99.5% of the 184 nodules they investigated, and only one (0.5%) was later diagnosed with a malignancy. Similarly, Mittendorf and McHenry (102) found that initially benign cytology findings remained unchanged in 86.7% of the cases with follicular lesions; malignancies were found in 6.7%.

This discussion is important because it is part of the routine clinical monitoring of patients with suspicious US features and benign FNAB cytological results. Clinical US follow-up should be performed every 12 to 18 months (9,22,23); nonetheless, patients with initially benign cytological results and suspicious US findings have been found to have higher malignancy rates during repeated FNAB follow-up than patients without suspicious US findings (97). A consensus review by the American Association of Clinical Endocrinologists (AECA), the ATA and the European Thyroid Association revealed that 31% of the 166 specialists interviewed would order another FNAB 6 to 12 months after obtaining initially benign cytological results, regardless of the recommended guidelines (103), and that only 6% would request an immunohistochemical panel after indeterminate cytology. This indication has become increasingly consistent in HBME-1, Gal-3, and BRAFV600E immunohistochemistry studies (87,88).

The current clinical accuracy of these clinical and laboratorial variables (ultrasound or scintigraphy, cytology, and possible repeated FNAB studies) as malignancy predictors for thyroid nodules is still controversial.

## FINAL CONSIDERATIONS

The literature from the last five years has revealed new prospects for and trends in the approach to the diagnosis of thyroid nodules, with greater emphasis on US review and investigations of cytological tumor markers. An US review combined with cytological data (including the Bethesda classification system) improves the accuracy and efficiency of thyroid nodule malignancy prediction in cases with indeterminate cytology, especially when reviewed by thyroid pathology experts. In most of the published studies, the use of ultrasound criteria to determine whether to perform repeated FNAB-US studies for nodules with initially benign cytology increased the diagnostic accuracy for malignancy over a mean follow-up of 12-18 months. Higher malignancy rates have been observed in

initially benign nodules with suspicious US findings than in those evaluated using the widely used nodule growth criteria.

The use of US malignancy criteria combined with Bethesda categories III or IV improves malignancy detection, and they should be considered to guide surgical decisions for this group of nodules.

Determination of the clinical applicability of genetic and molecular markers in FNAB samples requires additional, consistent long-term studies. The initial results presented in this field of FNAB immunohistochemical markers, were satisfactory for making decisions about which patients required surgery or clinical-follow up in specific cases, especially those with indeterminate cytology. The BRAF V600E mutation and the simultaneous cytological expression of HBME-1, Gal-3, and CK-19 improve malignancy prediction and are good candidates for guiding surgical decisions for Bethesda category III and IV nodules.

Malignancy prediction models are increasingly desirable for establishing early diagnoses and improving surgical decisions in specific patients, such as those with indeterminate or undiagnosed cytology thyroid nodules.

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## AUTHOR CONTRIBUTIONS

Maia FF conducted the cytopathological review, ultrasound and database searches and participated in the design and statistical analysis. Zantut-Wittmann DW conceived of the study and participated in the design and coordination. All of the authors read and approved the final version of the manuscript.

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