

# Evaluation of the Appropriateness of Thyroid Fine-Needle Aspiration

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## Abstract

*This study aims to perform an integrative literature review on FNAB of the thyroid and its current contributions facing thyroid nodules, as an important diagnostic tool to eliminate early thyroid cancer, a recurrent pathology in offices. Regarding the methods, because this is an integrative review of literature, through a descriptive study, we used databases such as Medical Literature Analysis and Retrieval System Online (Medline/PubMed), Scientific Electronic Library Online (SciELO) and Virtual Health Library (VHL). The query was performed using the controlled descriptors from DeCS/MeSH: "thyroid nodule", "fine needle biopsy", "thyroid", "liquid-based cytology", "molecular test". Moreover, as a result we obtained a general review about the appropriateness of FNAB of the thyroid, an efficient and reliable diagnostic method in the decision and selection of patients who will need surgery. However, this method presents some limitations, such as the need for improvement in the quality of the sample collection or in its interpretation, limitations that can be reduced, decreasing the cases of false positives and negatives, when collected by experienced professionals, guided by ultrasound, and can be combined with color Doppler, as well as, seek opinions from other specialists. The present study also addressed the new diagnostic methods for thyroid nodules, such as liquid-based cytology, used as a technique in conjunction with conventional smears when ancillary techniques are applied, and molecular tests, which have given professionals the opportunity to provide a more accurate evaluation in cases of indeterminate nodules. With this, we reiterate the importance of identifying the best individualized strategy for the patient, preventing unnecessary procedures and offering improved quality of life.*

**Keywords:** thyroid nodule; fine needle biopsy; thyroid; liquid-based cytology; molecular testing;

## 1. Introduction

The thyroid nodule constitutes a frequent pathology in today's society and is configured as the main manifestation of a number of thyroid diseases (Morais *et al.*, 2019). The incidental discovery of generally small and asymptomatic nodules in imaging exams, requested for reasons often unrelated to the thyroid, has collaborated to the high number of diagnoses (Forneiro *et al.*, 2022).

Although most thyroid nodules are benign, the diagnostic approach aims to exclude malignancy, since in some individuals, thyroid neoplasm can constitute an aggressive pathology (Morais *et al.*, 2019). The diagnostic evaluation of the nodule is extremely important to differentiate benign from malignant nodules,

through clinical characteristics, initial and cytopathological examinations, investigating mainly the individuals with greater suspicion of malignancy, aiming to provide the patient with an early, safe and appropriate treatment, thus reducing the number of aggressive cases (Silveira & Silveira, 2018).

Among the diagnostic tests used in thyroid nodules, the fine-needle aspiration biopsy (FNAB) of the thyroid stands out. The FNAB of the thyroid is a simple and safe outpatient procedure, minimally invasive, in which tissue samples are obtained for cytological examination and/or molecular tests. For the analysis, the Bethesda classification is used, which has six categories according to the risk of malignancy (Vilela & Carvalho, 2019).

This test is the best method to differentiate malignant and benign nodules, having been used as the gold standard, proving to be safe, easy to perform, low cost, low complication rate and with good accuracy, exhibiting high rates of sensitivity and specificity, therefore, its importance in the diagnosis of benignity or malignancy of thyroid nodules is highlighted. It is important to highlight that the use of an appropriate technique, experienced doctors to perform the FNAB and to analyze the aspirated material are determining factors in increasing the accuracy of the method, guiding an appropriate therapy (Morais *et al.*, 2019).

Moreover, a risk stratification system known as TIRADS was proposed in 2017 by the American College of Radiology, being very important to select patients who should undergo a biopsy by the fine-needle aspiration method, to reduce the number of unnecessary procedures, since most of the punctured nodules are benign. According to this classification, we can categorize nodules into benign, minimally suspicious, moderately suspicious, and highly suspicious (Silva & Ferreira, 2019).

This study aims to perform an integrative literature review on FNAB of the thyroid and its current contributions facing thyroid nodules, as an important diagnostic tool to eliminate early thyroid cancer, a recurrent pathology in offices.

## **2. Theoretical framework**

This theoretical reference will work on seven topics: a) the thyroid gland and thyroid nodules; b) diagnosis of thyroid dysfunctions; c) the importance of FNAB in the evaluation of thyroid nodule; d) how the thyroid FNAB is performed; e) cytopathological analysis according to the Bethesda System; f) liquid-based cytology and molecular tests.

### **2.1 The thyroid gland and thyroid nodules**

The thyroid is an endocrine gland of the human body which is located below the larynx, in the anterior part of the neck and below a region called Adam's apple, weighing on average 20 to 30 grams. It is responsible for the production of hormones, known as thyroid hormones, they are T3 (tri-iodothyronine) and T4 (thyroxine), extremely important for the proper functioning of the body (Porto, 2019, p. 700).

Several disorders can affect the thyroid, among them, the appearance of nodules in this region, considered one of the most frequent changes in the gland, since it can appear in almost all thyroid diseases. This appearance is due to areas of exaggerated growth that end up differentiating themselves from the rest of the thyroid tissue. Considering the high frequency in the general population, it is very important to define the

thyroid nodules that have a higher risk of developing cancer (Ross *et al.*, 2021).

Thyroid nodules can consist of cysts, goiters, thyroiditis, adenomas or carcinomas. As for malignancy, thyroid nodules can be classified as benign, malignant and indeterminate. The benign nodules are usually found in routine examinations and incidental findings, which, smaller or equal to 1 cm, the term incidentaloma applies. The indeterminate nodules are those that have benignity or malignancy well clarified, and in relation to malignant nodules, are usually more apparent in individuals with immunosuppression, and the most frequent are the papillary microcarcinomas (Filho, 2016, p. 1774).

### **2.2 Diagnosis of thyroid dysfunctions**

For initial evaluation, the anamnesis and physical examination of the thyroid are essential steps. The physical examination of the thyroid is the main tool in the diagnosis and evaluation of a patient with possible disorders in the gland, because it evaluates its aspects, through inspection, palpation and auscultation, noting the presence of likely changes, such as the presence of a nodule. Once the nodule is discovered, the doctor will determine if the nodule is the only problem or if the entire thyroid gland has been affected (Ross *et al.*, 2020).

Followed by the anamnesis and physical examination, the evaluation of the thyroid requires laboratory tests, which assess the function of the gland. Functioning is assessed by the dosage of thyroid-stimulating hormone (TSH), which is responsible for regulating the secretion of the hormones T3 and T4. However, direct measurement of these hormones remains important in many patients, as it may be difficult in some to be certain about the state of pituitary and hypothalamic function with TSH dosage alone (Ross *et al.*, 2021).

In cases of changes on physical examination and laboratory test results, more accurate evaluation includes other specialized tests, such as imaging tests and fine needle biopsy. Among imaging tests, the thyroid ultrasound is the most commonly used test, followed by thyroid scintigraphy (Vilela & Carvalho, 2019). Computed Tomography and Magnetic Resonance Imaging are rarely indicated, as they do not differentiate malignant and benign lesions, but are useful in the evaluation of diving goiters or the compression and invasion of adjacent structures, such as the trachea (Morais *et al.*, 2019).

Thyroid US is the most commonly indicated exam and used to confirm physical examination findings, and to guide FNAB, a gold standard test in distinguishing benign and malignant nodules (Morais *et al.*, 2019). It assesses the anatomy of the gland, determining the exact size of the nodule, whether a nodule is solid or cystic, whether it has calcifications, and whether they are growing or decreasing (Vilela & Carvalho, 2019).

Thyroid scintigraphy is used to determine the functional status of a nodule, and can be useful in patients with multiple thyroid nodules to select those that are hypofunctional or hyperfunctional. It is indicated if the TSH dosage is elevated (Ross *et al.*, 2020).

### **2.3 The importance of FNAB in the evaluation of thyroid nodule**

The FNAB of the thyroid is a method of fundamental importance in the diagnosis of thyroid neoplasms and is the gold standard because it differentiates benign and malignant nodules. It is an easy test to perform, with high sensitivity and specificity, low invasive, low cost, low complication rate and good accuracy. It also

has low false positive and false negative rates, reducing unnecessary thyroidectomies. It requires the use of an appropriate technique, experienced physicians to perform the procedure and to analyze the aspirated material, being determining factors in increasing the accuracy of the method, guiding an appropriate therapy (Morais *et al.*, 2019).

Moreover, the importance of FNAB of the thyroid also consists in the fact that before its implementation, thyroid nodules were routinely referred to diagnostic surgery, an invasive method, due to the possible risk of malignancy. As such, FNAB has decreased the incidence of diagnostic thyroidectomies, as most results from aspirated material are conclusively diagnosed as benign cytology (Morais *et al.*, 2019).

### **2.4 How the thyroid FNAB is performed**

In this test, a small needle (23 to 27 caliber, commonly 25 caliber) is introduced into the nodule in order to extract the cell samples, that will later be analyzed. It is performed with or without lidocaine or xylocaine anesthesia, by passing a needle through the nodule. The needle is attached to a 10 ml syringe, which can be contained in a holder designed to facilitate the application of constant or intermittent suction. Ultrasound is used to pinpoint the exact location where the procedure is to be performed. The aspirated material is stained directly on blades, fixed or can also be collected in a liquid preservative, from which thin-layer preparations are made (Ross *et al.*, 2022).

### **2.5 Cytopathological analysis according to the Bethesda System**

The result of the material collected in the puncture is classified according to the analysis of cytopathology. This analysis is called the Bethesda System, which guides thyroid cytopathology reports by dividing them into 6 categories based on the risk of malignancy, being: I-Non-diagnostic sample; II-Benign; III-Atypia/follicular lesion of undetermined significance; IV-Suspect for follicular neoplasm or follicular neoplasm; V-Suspect for malignancy; and VI-Malignant (Morais *et al.*, 2019).

The Bethesda System is also intended to direct the type of treatment. Surgical treatment is recommended for patients whose cytology is suspicious for malignancy or malignant (category V or VI). When the cytology is suggestive of follicular neoplasm (category IV), the radioiodine scintigraphy should define the management, because if the nodule is hyperuptake, its removal is not necessary; when the nodule is hypocaptant, surgery is indicated. If the cytology suggests follicular lesion or atypia of undetermined significance (category III) or unidentified sample (category I), a new puncture should be done with an interval of 3 to 6 months. If this result persists, surgery is indicated in patients with a high clinical or sonographic suspicion of malignancy or a nodule larger than 2 cm. And finally, if the sample is classified as benign (category II) but the nodule marks a combination of sonographic findings suspicious for malignancy, associated with risk factors, repeat puncture is advised (Morais *et al.*, 2019).

### **2.6 Liquid-based cytology and molecular testing**

Liquid-based cytology (LBC) is a monolayer slide preparation method that surpasses conventional smear (CS) examinations due to more accurate fixation with reduced obscuration conditions and standardized cell

transfer. In LBC, the sample is collected by fully immersing the sampling device into the vial of preservative site fluid, which simultaneously preserves and fixes the cells, not so in conventional smears, where the sample is spread on a glass slide and fixed separately. (Makde & Sathawane, 2022).

Furthermore, molecular testing improves the diagnostic accuracy of indeterminate thyroid nodules, and provides relevant information about tumor prognosis. They are able to identify molecular variants and fusions, and are useful for informing physicians regarding a patient's eligibility for targeted systemic therapies, such as in the case of thyroid cancer with metastatic, progressive, radioiodine-refractory disease (Patel *et al.*, 2023).

### **3. Methodology**

The present study is an integrative literature review, carried out from March 2022 to April 2023. To this end, an order was followed for the production of the work, which was: definition of the theme to be addressed; search and choice of descriptors in the database; definition of criteria for inclusion and exclusion of articles; search and selection of articles in the literature; critical analysis of the acquired content; discussion of results and, finally, construction of the review.

Moreover, this research was operationalized through the Population, Intervention, Comparison, Outcome (PICO) strategy, which serves as a guide for the construction of research, and from this, the guiding question of this study was developed: How is the appropriateness of fine needle aspiration puncture in thyroid nodules?

For the search for data, the search was performed in electronic databases: Medical Literature Analysis and Retrieval System Online (Medline/PubMed), Scientific Electronic Library Online (SciELO) and Virtual Health Library (VHL), due to their relevance in the literature and their large collections of complete documents. The query was performed using the controlled descriptors from DeCS/MeSH: "thyroid nodule", "fine needle biopsy", "thyroid", "liquid-based cytology", "molecular test". Combinations were performed with the boolean operator AND, and all the keywords used were cross-referenced.

Moreover, the following inclusion criteria were established: articles related to the theme in question, published between 2018 and 2023, with full text, in English, Spanish and Portuguese, indexed in one of the databases cited above. The exclusion criteria were: articles that did not present the main theme, articles that did not answer the guiding question, duplicate articles, those that did not cover the full text, those published outside the interval from 2018 to 2023, and those that did not address the languages selected. After the search, its quality and relevance were analyzed from the abstracts, and since then, the journals more congruent with the theme were selected, where the presentation of the data was recorded in a table, presenting: year, author, title, and main conclusions.

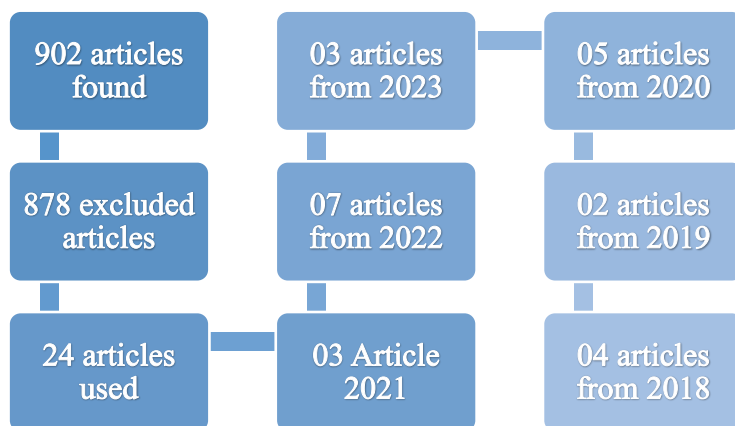
### **4. Results and discussion**

After starting the search for articles, a total of 902 articles were found. Soon after, we read the full text of 62 articles, which stood out for some specific criteria, namely: diagnostic features of FNAB of the thyroid, its suitability and contribution to the cytopathological examination, as well as molecular tests. However, of these 62 articles, 24 were selected for the basis of the study. Thus, 878 were excluded for not fully fit for discussion against the diagnosis through of thyroid FNA. Finally, of the articles used to help in the writing of this paper,

03 comprised the year 2023, 07 comprised the year 2022, 03 the year 2021, 05 the year 2020, 02 the year 2019 and 04 the year 2018, as shown in figure 1.

**Figure 1**

*Flowchart of the article search*



*Note.* Source: The authors, 2023.

During this study, 24 articles were chosen to be examined. Chart 1 comprises the articles used for the integrative literature review on the theme proposed in the paper, from the analyzed databases.

**Chart 1**

*Articles used for the integrative literature review on the theme proposed in the work*

YEAR	AUTHOR	TITLE	MAIN CONCLUSIONS
2022	Guindan, <i>et al.</i>	Valor de la PAAF y el Sistema Bethesda en tumores de tiroides	FNAB and the report according to the Bethesda system is reliable for assessing the malignancy of thyroid nodules and classifies the need for surgical treatment to the patients studied preoperatively.
2023	Joo, <i>et al.</i>	Diagnostic performance of ultrasound-based risk stratification systems for thyroid nodules: a systematic review and meta-analysis	False-positive FNA results imply a risk of unnecessary biopsies and the TIRADS classification can help in reducing the number of these biopsies.
2022	Foroughi, <i>et al.</i>	Concordance between TIRADS and Cytology in	There is relevant agreement between TIRADS and cytology in patients with thyroid nodules.



		Thyroid Nodule	
2018	Cordes, <i>et al.</i>	Fine-needle aspiration biopsies of thyroid nodules	Adverse events were rarely observed, and those that were, did not require intervention or could be self-managed by the patient.
2023	Park, <i>et al.</i>	A comprehensive assessment of the harms of fine-needle aspiration biopsy for thyroid nodules: a systematic review	It is important to perform a complete evaluation of the patients' medical condition when deciding to perform FNA, as it decreases possible complications.
2020	Pinto-Blázquez, Jesús & Úrsua-Sarmiento.	Anatomía patológica de la patología de tiroides y paratiroides. Sistema Bethesda del diagnóstico citológico de la patología de tiroides	The Bethesda System is a classification that gives more homogeneous reports that allow the best therapeutic management to be established for the patient.
2018	Brites, <i>et al.</i>	Investigation of correlation between cytological and histological findings in suspected carcinoma of thyroid	Malignancy rates were higher in Bethesda III. The association between Bethesda II category and thyroid carcinoma was lower than expected.
2022	Rodríguez-González, <i>et al.</i>	Cómo mejorar la precisión de los diagnósticos I y III del Sistema Bethesda	Nowadays there are methods to obtain better results in inconclusive cases of FNAB. When the samples are unsatisfactory, a surveillance attitude is recommended, depending on the ultrasounds results.
2019	Núñez & Vela	Eficiencia de la punción por aspiración con aguja fina en el diagnóstico de las afecciones del tiroides	Despite being considered the most reliable method for selecting patients to undergo surgery, this study revealed limitations of FNAB for definitive diagnosis in thyroid nodules.
2022	Wang, <i>et al.</i>	Grayscale, subjective color Doppler, combined grayscale with subjective color Doppler in predicting thyroid carcinoma: a retrospective analysis	Gray-scale ultrasound associated with subjective color Doppler and ultrasound-guided per FNAB biopsies are recommended for the diagnosis of thyroid carcinoma.
2020	Pantanowitz, <i>et al.</i>	Is thyroid core needle biopsy a valid compliment to fine-needle aspiration?	Core needle biopsy (CNB) of the thyroid gland may demonstrate a good tool to complement the diagnosis when the initial

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			outcome was inadequate or indeterminate FNA.
2020	Poller <i>et al.</i>	Measures to reduce diagnostic error and improve clinical decision making in thyroid FNA aspiration cytology: A proposed framework	Based on the authors' personal experience, 12 recommendations have been portrayed to aid clinical decision making, ensure quality, and reduce diagnostic error in FNAB and histopathology of the thyroid.
2021	Ucak, <i>et al.</i>	Evaluation of thyroid fine-needle aspiration biopsies according to cytological methods and comparison with histopathological diagnosis	It is recommended to use the liquid-based cytology (LBC) method, which seems to have the highest sensitivity, rather than the conventional smear (CS).
2022	Sharma, <i>et al.</i>	Diagnostic utility of conventional and liquid-based cytology in the management of thyroid lesions; an institutional experience	The LBC method allows the storage of a variable number of cells for up to 6 months after FNAB, so the material remaining in the vial can be used for the application of ancillary techniques such as immunohistochemistry, flow cytometry and molecular biology.
2022	Makde & Sathawane	Liquid-based cytology: Technical aspects	Two main methods of liquid-based preparation are known, which are ThinPrep and SurePath. These two methods differ in their collection principles, yet they produce similar preparations.
2022	Sayer, <i>et al.</i>	Comparison of Conventional Smear and Liquid-Based Cytology in Adequacy of Thyroid Fine-Needle Aspiration Biopsies without an Accompanying Cytopathologist	The adequacy rate of FNAB was significantly higher in the LBC method compared to the CS method, the former being more practical and faster.
2021	Mahajan, <i>et al.</i>	Should liquid based cytology (LBC) be applied to thyroid fine needle aspiration cytology samples?: comparative analysis of conventional and LBC smears	Overall, CS's were better in their diagnostic efficacy. Therefore, LBC should be viewed as a complementary technique if ancillary techniques such as immunohistochemistry or molecular testing are needed.



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2018	Sharma, <i>et al.</i>	Cytomorphological differences between liquid-based cytology and conventional smears in fine-needle aspirates of thyroid lesions	The LBC can complement the CS, but it cannot replace it.
2020	Kant, <i>et al.</i>	Thyroid Nodules: Advances in Evaluation and Management	Molecular testing identifies mutations related to thyroid cancer and can help inform decisions about surgical excision versus continuous ultrasound monitoring.
2019	Angell.	Getting more out of molecular testing for indeterminate thyroid nodules	Cytological evaluation and, more recently, molecular testing have refined the ability of doctors to provide the most accurate assessment of the risk of malignancy.
2021	Patel, <i>et al.</i>	Molecular testing for thyroid nodules including its interpretation and use in clinical practice	Molecular testing has been incorporated as an important diagnostic adjunct in the treatment of indeterminate thyroid nodules in the American Thyroid Association (ATA) guidelines.
2018	Nishino & Nikiforova.	Update on molecular testing for cytologically indeterminate thyroid nodules	The 4 commercially available molecular tests for thyroid cytology offer unique approaches to improve risk stratification of thyroid nodules.
2023	Patel, <i>et al.</i>	Molecular diagnostics in the evaluation of thyroid nodules: Current use and prospective opportunities	In the last 15 to 20 years, there have been rapid advances in cytomolecular testing to aid in the treatment of thyroid nodules.
2020	Grani, <i>et al.</i>	Contemporary thyroid nodule evaluation and management	When multiple diagnostic and/or treatment options exist, they should be discussed with patients as frankly as possible to identify an approach that best meets their needs.

*Note.* Source: The authors, 2023.

FNAB is an indispensable diagnostic tool to categorize the evolution of thyroid nodules and useful to organize the most appropriate management and identify patients susceptible to surgical intervention. In addition to being a safe, low-cost, easy-to-perform procedure, FNAB has a high sensitivity, that is, few false negative rates (Guindan *et al.*, 2022). According to Joo *et al.* (2023), false-positive results imply a risk of

potential complications and increased costs due to the fact that more biopsies are required. In addition, inconclusive results can lead to unnecessary surgical approaches for some thyroid nodules.

According to Foroughi *et al.* (2022), there is still significant agreement between cytology and imaging techniques for better thyroid nodule detection. Ultrasonography increases the detection rate of benign nodules and nodules suspected of malignancy, and TIRADS can be used to decrease the number of unnecessary punctures in apparently benign nodules, since suspicious nodules found on ultrasonography should undergo FNAB.

The thyroid FNAs are mostly safe, once the requirements of technique and hygiene are obeyed. Although it is a well-tolerated method among patients submitted, they can still be affected by some complications that can be contained, not requiring medical intervention. Among these adverse events are pain, presence of hematomas, bleeding, and more rarely infections (Cordes *et al.*, 2018).

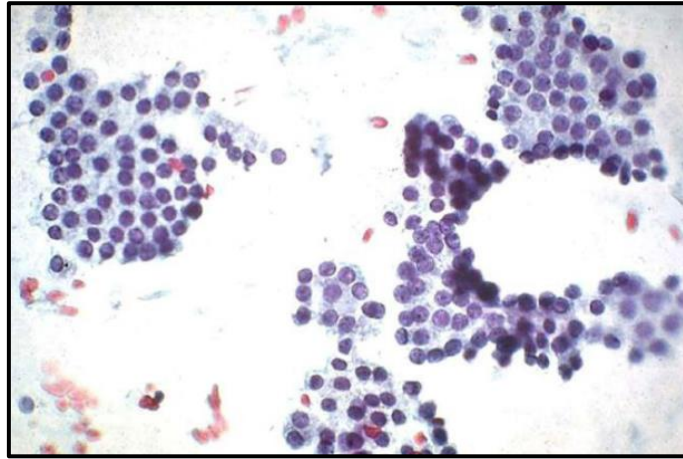
Based on these complications, the study by Park *et al.* (2023) reports some ways to reduce these adverse events, such as: use smaller caliber needle, perform local anesthesia in patients more sensitive to pain; analyze propensity for bleeding, discontinue anticoagulants depending on the case; do Doppler ultrasound in the search for high vascularity; have redoubled attention when elderly patients, because there is a greater chance of venous fragility and possible arteriovenous shunts; avoid puncturing the dorsal part of the nodule to avoid damaging the vocal cords; perform a sterile procedure, especially in immunocompromised patients and if suspected, start empirical antibiotic treatment and drainage; finally, perform thyroid function tests in suspected post-aspiration thyrotoxicosis.

The analyses obtained through FNAB are classified according to the Bethesda system, a scale developed to differentiate samples with a higher probability of malignancy and which is divided into 6 diagnostic categories that will guide the management of the patient with a thyroid nodule (Guindan *et al.*, 2022).

Category I of the Bethesda system concerns inconclusive nodules, with a risk of malignancy between 5-10%. To reach this conclusion, a histological parameter of 6 groups with at least 10 well visualized and preserved follicular cells must be observed to be considered satisfactory, as shown in figure 2. Unsatisfactory samples constitute less than ideal number, low quality (intense artifact), samples with blood material, muscle or respiratory-like cylindrical cells, as well as the absence of follicular cells. There is an exception for solid nodules with atypia on FNAB, solid nodule with inflammation and samples with abundant colloidal material, because in these cases, the nodules are not considered inconclusive, even if the amount of clusters and follicular cells is lower than estimated. In this category it is recommended to repeat FNAB with ultrasound control (Pinto-Blázquez & Úrsua-Sarmiento., 2021). Although it represents a clinical challenge, according to Brites *et al.* (2018), category I is more likely to present benign than malignant results.

### **Figure 2**

*FNAB of benign follicular nodule showing clustering of follicular cells*

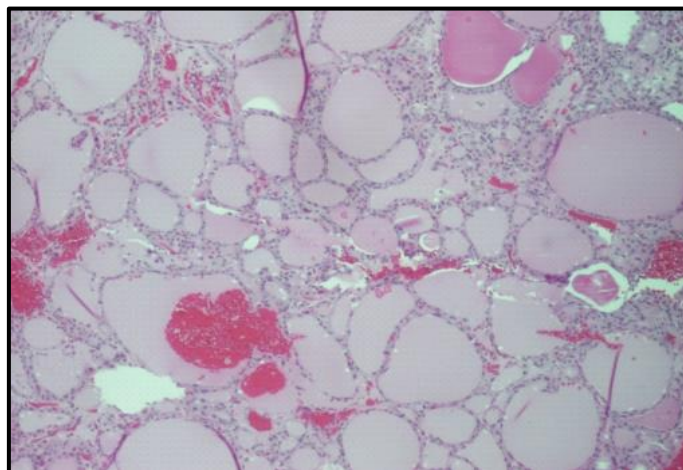


*Note.* Source: Pinto-Blázquez (2021).

About 60-70% of thyroid nodules are benign and fall into the Bethesda category II. The risk of malignancy varies only between 0 and 3%. The benign follicular nodule is the most common form. Other examples are lymphocytic thyroiditis, granulomatous thyroiditis, acute thyroiditis and Riedel's thyroiditis. Histologically, distension of large thyroid follicles can be observed, demonstrated in figure 3. In these cases only ultrasound and clinical follow-up is indicated (Pinto-Blázquez and Úrsua-Sarmiento, 2021).

**Figure 3**

*Histological sample of multinodular goiter showing several distended follicles*



*Note.* Source: Pinto-Blázquez (2021).

Category III refers to atypia or follicular lesion of uncertain significance and comprises a heterogeneous group of lesions from the histological point of view, with a risk of malignancy between 10 and 30%. In this group, samples with architectural or cytological irregularities greater than expected from a benign sample are observed, but at the same time they are insufficient to be classified as malignant. The presence of histiocytoid

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cells, punctures with small oncocytic cells, and unspecified atypia may be observed. The Bethesda system recommends repeat FNAB in these cases (Pinto-Blázquez & Úrsua-Sarmiento, 2021).

Category IV includes follicular neoplasia or suspicious follicular neoplasia, and in these cases the Bethesda system recommends lobectomy or molecular techniques, since FNAB cannot distinguish adenoma from carcinoma. The V category, on the other hand, includes samples highly suspicious for malignancy, such as lesions suspicious for papillary or medullary carcinoma, lymphoma or other unspecified lesions, but suspicious for malignancy, and in these cases, the Bethesda system recommends lobectomy or thyroidectomy (Pinto-Blázquez & Úrsua-Sarmiento, 2021).

Bethesda category VI includes cytological findings conclusive of malignancy such as papillary carcinoma and variants, medullary carcinoma and variants, anaplastic carcinoma, poorly differentiated carcinoma, lymphoma and metastasis. In these cases, it is necessary to investigate metastases and see the need to perform total/partial thyroidectomy or lobectomy (Pinto-Blázquez & Úrsua-Sarmiento, 2021).

According to Rodríguez-González *et al.* (2022) categories I and III represent a challenge in terms of clinical management because some patients, especially in category III, undergo diagnostic surgery, such as lobectomy, when they do not need it. Thus, it is necessary to seek strategies to avoid trauma to the patient and reduce health costs that the excess of procedures implies. One strategy would be to repeat the puncture recommended by the Bethesda system itself, since 10 to 30% of nodules are reclassified as category III after the second puncture, which would avoid diagnostic lobectomy. Other strategies would be to increase the clinical value of the samples when they are accompanied by echographic findings with a high risk of malignancy as in the TIRADS 5 category, and to associate them with immunohistochemical and molecular studies.

According to the study by Núñez & Vela (2019), even though FNAB is the most efficient and reliable method for deciding and selecting patients who will undergo surgery, the method revealed limitations for the purpose of definitive diagnosis of thyroid pathology. In this research, FNAB results were compared with paraffin block biopsy results, which demonstrated discrepancies between FNAB and paraffin block biopsy diagnoses. Thus, a mathematical analysis of the obtained results was performed, which specified that the sensitivity and specificity of FNAB were not sufficient to close a diagnosis.

However, most researchers report a diagnostic efficacy of FNAB higher than 85%, and when performed by specialists it is higher than 95%, and in this study it was 80%, which may be indicative of the need to improve the quality of sample collection or its interpretation, since obtaining adequate cell smears is essential to optimize this performance (Núñez & Vela, 2019). Therefore, analysis of satisfactory samples, collected by experienced professionals can reduce the diagnostic limitations of FNAB.

Additionally, the study developed by Wang *et al.* (2022) revealed that the disadvantages of FNAB of the thyroid are the false-negative and false-positive results. However, the combination of grayscale ultrasound with fine needle aspiration biopsy, guided by subjective color Doppler ultrasound, can help in the definitive diagnosis, reducing the cases of false positives and negatives, being recommended in cases of suspected thyroid carcinoma.

Moreover, studies show that core needle biopsy (CNB) of the thyroid may be a good complementary

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diagnostic tool in individuals who present with inadequate or indeterminate initial result of FNAB. This complementation is advantageous because the CNB can seize larger amounts of tissue, make the evaluation of histological architecture, as well as can perform immunohistochemistry in a more accessible way when it is necessary. However, in this method there is a greater chance of pain, bleeding, displacement of tumor cells and difficulty in approaching certain lesions posterior to the thyroid gland or near vital structures such as the carotid artery or trachea. Thus, it is interesting to remember that the American Thyroid Association (ATA) does not recommend its use in thyroid tumors (Pantanowitz *et al.*, 2020).

In view of this, regarding the prevention or reduction of misdiagnoses, Poller *et al.* (2020) listed some suggestions for this, such as: understand and explain to patients the limitations of the procedure; always expose the risk of malignancy according to the result; if possible, perform a quick analysis of the aspirate at the site; make the analysis of clinical and sonographic findings; seek the opinions of other specialists, from the same or another institution. They also recommended a multidisciplinary discussion about the therapy, as well as properly manage the expectations of the patient and the clinician regarding the cytological analysis of the thyroid nodule.

In relation to the clinical practice setting, the aspirated contents are prepared by conventional smear (CS) methods, which consist of examining the cells by spreading directly on the blade (Ucak *et al.* 2021). However, these conventional preparations commonly contain some disadvantages, such as a dirty and hemorrhagic background, which can make it difficult to make a diagnosis. Appropriateness creates concern, especially when the lesion is hemorrhagic, and with this in mind, liquid medium preparations have emerged (Sharma *et al.*, 2022).

Authors have reported that the liquid-based cytology smear (LBC) technique has been widely used for thyroid FNA, as shown in figure 4, a method in which cells are placed in a solution in a special tube and the preparation is created in an automated device (Ucak *et al.* 2021). Currently, two main liquid-based preparation systems are on the market: the ThinPrep and SurePath. These methods have different cell harvesting regimes, but very similar preparation yields (Makde & Sathawane, 2022).

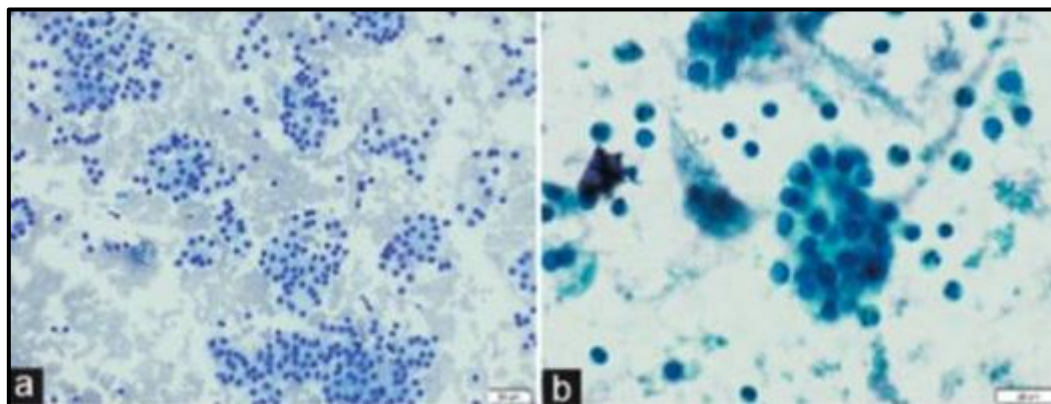
According to Sayer *et al.* (2022), LBC becomes feasible for pathologists because it features shorter preparation time, ease of transport, elimination of artifacts, can provide the opportunity for additional preparations from the sample, more details in evaluating a small area and in large numbers of cells, making it easier to detect malignancies, and also, there is no need to learn the smear technique. Furthermore, given the cytological diagnosis, the rest of the collected material can be used for applications of ancillary techniques such as immunohistochemistry, flow cytometry and molecular biology. This is because the LBC method can preserve varied numbers of cells for up to 6 months after FNAB (Sharma *et al.*, 2022).

In contrast, in a study by Mahajan *et al.* (2021), no superiority was found with the LBC method in FNAB of the thyroid, on the contrary, it was reported higher efficacy related to cellularity and diagnosis in CS, recommending that CS should not be replaced in clinical routine, but it can be complemented when ancillary techniques are needed, such as immunohistochemistry or molecular tests, thus corroborating with the study of Sharma *et al.* (2018).



**Figure 4**

*Conventional smears (a) versus liquid cytology (b) in a representative case of follicular neoplasia*



*Note.* Source: Adapted from Mahajan *et al.* (2021).

Giving segment with the updates in front of thyroid FNA, molecular testing (MT) has transformed the way in which thyroid nodules with indeterminate cytology are approached. Studies show that a pattern of benignity in these tests greatly reduces the risk of malignancy, and that they have enhanced the ability of physicians to grant a more accurate assessment of this risk (Kant *et al.*, 2020; Angell, 2019). The ATA indicates that, after the analysis of clinical and ultrasound characteristics, MT can be used together to determine the risk of indeterminate thyroid nodules (Patel *et al.*, 2021).

MTs aim to spare patients from undergoing unnecessary surgery for benign nodules, differentiate high risk cancers that are candidates for total thyroidectomy or low/intermediate risk cancers that have lobectomy as their initial choice. These tests are based on 3 main performances: gene expression assessment, microRNA-based classifiers and somatic mutation testing. Currently, 4 tests are available for thyroid FNA: Afirm, ThyroSeq, ThyGenX /ThyraMIR, and RosettaGX Reveal (Nishino & Nikiforova, 2018).

Therefore, MT is a tool that contributes in understanding the prognosis of those with specific mutations identified in thyroid cancer. Some mutations have been related to that of tumor aggressiveness, metastatic lymph node spread, propensity for dedifferentiation, and/or decreased efficacy of radioactive iodine therapy. The major known genetic causes of thyroid cancer include point mutations in BRAF, RAS, TERT promoter, RET and TP53 genes, as well as RET/PTC, PAX8/PPAR $\gamma$  and NTRK fusion genes (Patel *et al.*, 2023).

In view of this, the evaluation and management of thyroid nodule does not occur in a unique way for everyone, but it is up to the medical team to offer a personalized approach along with a careful analysis in determining the result, as well as clearly discuss the possible options with the patient, listing the risk factors, advantages and disadvantages of each one (Grani *et al.*, 2020).

## **5. Conclusion**

FNAB is the best test to detect the malignancy of a thyroid nodule, but despite being a safe, low cost, easy



to perform, and high sensitivity procedure, inconclusive results from samples or included in Bethesda category III can lead to a number of unnecessary punctures. The classification of the sample according to the Bethesda system guides the management of the patient with thyroid nodule, and the reduction of trauma to the patient and the health costs that the excess of the procedure implies, can be avoided through the repetition of the puncture only when it is recommended by the system. In addition, ultrasound increases the detection rate of benign nodules and nodules suspected of malignancy, and TIRADS can be used to decrease the number of unnecessary punctures in apparently benign nodules.

From the above, it is clear that the FNAB of the thyroid is an efficient and reliable diagnostic method for the decision and selection of patients who will undergo surgery, having as limitations the need for improvement in the quality of sample collection or interpretation, since obtaining appropriate cellular smears is essential for the analysis of satisfactory samples, limitations that can be reduced, reducing the cases of false positives and negatives, when collected by experienced professionals, guided by ultrasound, which may be combined with color Doppler, as well as, seek the opinions of other specialists.

Finally, this study addressed issues currently discussed about the new diagnostic methods for thyroid nodules, such as liquid-based cytology, used as a technique in conjunction with conventional smears when ancillary techniques are applied, such as immunohistochemistry or molecular tests. Furthermore, molecular testing has given practitioners the opportunity to provide a more accurate evaluation in cases of indeterminate nodules according to the Bethesda system. With this, we should identify the best individualized strategy for the patient, preventing unnecessary procedures and offering improved quality of life.

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