

# Histological Diagnosis of Thyroid Disease Using Ultrasound-Guided Core Biopsies

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## Key Words

Thyroid nodule · Ultrasonography · Core biopsy · Histological diagnosis · Hospital committee

## Abstract

**Background:** Thyroid core biopsies obtained with ultrasound (US)-guided needles are an alternative to conventional fine-needle aspiration and, according to various authors, have greater sensitivity and specificity. The technique is inexpensive, rapid and reliable with a low rate of complications, similar to conventional fine-needle aspiration procedures. **Objectives:** This paper critically reviews the methodology for obtaining samples and processing them in the pathology laboratory. **Methods:** Accumulated experience with 1,065 cases of US-guided core biopsy of the thyroid gland in a 15-year period. **Results:** US-guided core biopsy is a useful, inexpensive and safe method in the histological diagnosis of thyroid gland pathology. Thyroid samples obtained this way are not a substitute for fine-needle aspiration cytology. Indeed, some authors assert that the best results are obtained by combining the two approaches, the methods being complementary. **Conclusions:** To take best advantage of the findings from these techniques, pathologists must know which types of diag-

noses can be made and the fundamentals of how and, lastly, what cannot be diagnosed and the reasons why. Best results are obtained with a multidisciplinary approach in a hospital committee composed of endocrinologists, surgeons, radiologists and pathologists, who analyse and provide a background on each case.

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## Introduction

Radiological scans of the neck (ultrasound (US), computed tomography and magnetic resonance imaging), in many cases carried out for reasons unrelated to the thyroid gland, lead to the finding of many thyroid nodules, both palpable and non-palpable. Some of these nodules would never produce clinical signs or symptoms. Often nodules >1 cm in diameter are biopsied, the remaining being closely monitored by US. Around 3–6% of these nodules are neoplastic, the percentages being similar in palpable and non-palpable nodules. Recently, however, Liebeskind et al. [1] reported, in a series of 225 patients, a considerably higher percentage of carcinoma cases among nodules found incidentally, with a rate as high as 17%.

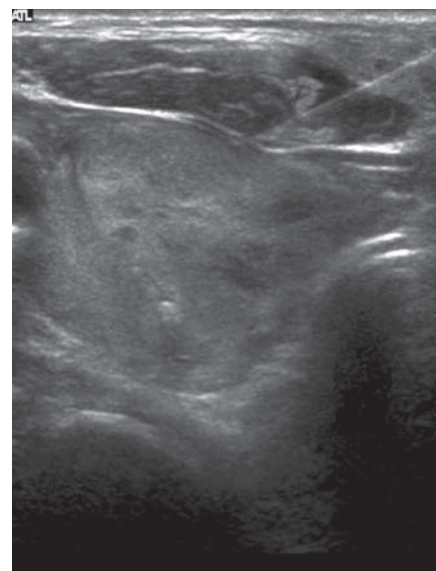
US is the radiological technique most widely advocated for assessing thyroid nodules since it is inexpensive, non-invasive, easy to perform and highly sensitive, and is included in most clinical protocols. It is certainly more accurate than palpation, detecting five times as many nodules [2, 3]. It does, however, have limitations; in particular, it has a low reliability for predicting malignancy. In this context, the combination of US and core biopsies followed by cytological and histological analysis seems to be more useful from the diagnostic point of view.

The use of US-guided core biopsies not only decreases the likelihood of sampling errors, but also allows one or several nodules to be properly assessed in the case of multinodular goitre, increases the sensitivity and specificity of the technique [4], and makes it possible to take samples of the solid areas in cystic lesions. Despite fine-needle aspiration cytology (FNAC) being almost universally used to take thyroid samples and, accordingly, there being extensive accumulated experience with the technique, in up to 20% of cases insufficient numbers of cells or non-diagnostic specimens are obtained, the rate varying between series. This figure can be even higher in lesions with a large cystic component, while taking a second biopsy reduces it by approximately 50% [5, 6].

Obtaining an US-guided core biopsy, rather than only cytological samples, is a technique that, although it is much less widely used, has been performed for more than a decade with better results than FNAC [7–11]. For example, Renshaw and Pinnar [10] analysed 377 patients who underwent both FNAC and US core biopsy and found statistically significant differences in the percentage of patients accurately diagnosed in favour of the core biopsy (82.2 vs. 70.3% with FNA), although these authors also recognised that the combination of the two techniques is the strategy that achieves the best outcomes (88.9%).

### Obtaining the Sample

Lesions should be sampled with US guidance. Exclusively palpation-guided biopsies have clear limitations compared to imaging-guided biopsies: they are less accurate for determining the access to the lesion, and do not make it possible to distinguish between parts of the lesion that are fluid-filled or solid (which are those to be sampled), between thyroid lesions and those in neighbouring tissue, or between focal lesions and diffuse lesions, such as thyroiditis, which do not need to be sampled. Accord-



**Fig. 1.** US image of multinodular hyperplasia. The needle can be seen at the edge of the largest node.

ingly, core biopsies guided by US are more reliable and lead to fewer false negatives than those relying only on palpation [12].

Before the biopsy, we should perform an initial scan to guide our selection of the best route of access. With the US-guided procedure, there are two options for guiding the needle: using an US probe device or a free-hand technique. The former option involves a device that is coupled to a transducer through which the needle inserted, following the fixed route previously marked on the skin. As for the free-hand technique, this consists of inserting the needle on one side of the probe, following the route indicated by the radiologist, while monitoring its path with the probe, held in the other hand, ensuring that the needle remains as parallel as possible to the axis of the transducer. This latter technique allows greater freedom, allowing the radiologist both to select the entry point and to adjust the route during the procedure, and is the approach the authors use (fig. 1).

For taking the tissue samples, the most useful devices are automatic, spring-loaded biopsy guns with cutting-edge needles. In these, the biopsy is taken by a core needle. When fired, the outer cannula is thrown beyond the central part of the needle cutting a sample of tissue which is then held inside it. The best of these guns have a variable throw length, to allow the radiologist to adjust the biopsy to the size and depth of the lesion. To take a tissue

sample, the needle is inserted until its tip is beside the nodule to be biopsied and the gun is fired, the needle being thrown the set distance and obtaining a cylindrical core. When possible, the needle is positioned to sample the edges of lesions.

To select the lesions to biopsy, the authors apply the criteria of the Washington consensus [13] with small modifications. Specifically, where measurements refer to the largest diameter, we take biopsies of all calcified lesions >1 cm, solid lesions >1 cm, and those with mixed cystic and solid components >1.5 cm, including cystic lesions with mural nodules. Further, we recommend that any lesions that have significantly grown since the previous check-up should be biopsied, except in the case of exclusively cystic lesions. In order to take a biopsy from lesions with a significant cystic component, first the fluid within the cyst is removed and processed for cytological analysis, and then a core biopsy is taken of the nodule. Finally, a biopsy is also indicated if the radiologist detects suspicious lymph node changes (enlargement or microcalcification).

### Sample Processing

The core biopsy samples obtained should be processed in a way that maximises the information obtained. Generally, few samples are obtained, two or three at best, usually containing both normal and pathologic tissue. Further, since the thyroid is an organ with relatively thin stromal tissue, samples are fragile and tend to collapse, thyroid follicles breaking easily. Accordingly, the material must be handled very carefully, taking particular care to damage the sample as little as possible when removing it from the transport medium.

If saline solution is used as the transport medium, low-speed centrifugation yields a cytological sample that may be very useful to assess the nuclei. Refrigeration of the transport medium will prevent tissue autolysis and contribute to maintaining the samples in optimal conditions for diagnosis. We have already successfully applied this approach with similar samples from other sites [14–17]. Alternatively, it is possible to use a fixative fluid from liquid-based cytology.

Biopsy core samples are then processed in the routine manner, fixed in formalin and embedded in paraffin. Paraffin block slices should be taken from the beginning with the microtome, keeping two or three unstained slides mounted for immunohistochemistry, if necessary.

### What Can Be Diagnosed and How

It is useful for the pathologist to first analyse surgical specimens of thyroid tissue with common diseases such as goitre, thyroiditis, and papillary thyroid carcinoma, as well as ‘histologically normal’ tissue, analysing them under high magnification to simulate the conditions of tissue scarcity to be encountered with the core biopsy specimens.

#### *Normal Thyroid Tissue*

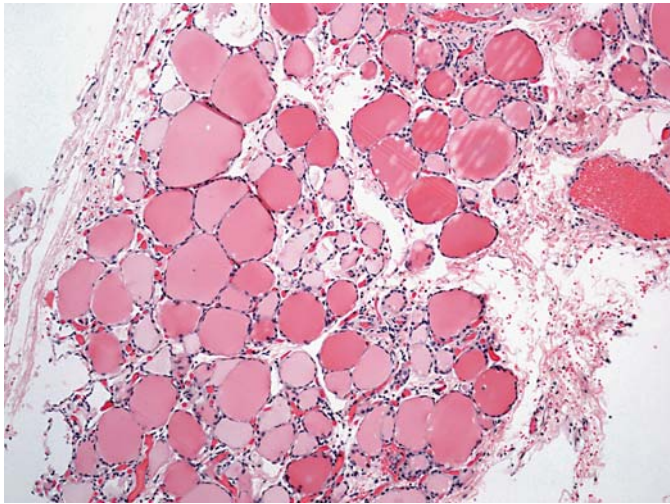
It is not common to take biopsies of the thyroid if cysts, nodules, tumours or other types of lesions have not been detected. Accordingly, normal tissue tends only to be obtained when the radiologist misses the target lesion. This can occur due to lack of experience or to intrinsic difficulties such as in the case of small nodules or when they are located in hard-to-reach areas. We can diagnose thyroid tissue as normal or without significant changes, when the core biopsy samples have regular follicles, with no folding or other signs of thyroid hyperfunction, and a non-smooth surface, similar to an underinflated beach ball (fig. 2), within a stromal matrix that is thin or unnoticeable and there are no signs of fibrosis, inflammation or haemorrhage. The follicular cells are cuboidal and the nuclei are small and hyperchromatic.

#### *Hyperplastic Thyroid Tissue*

The most common finding on thyroid biopsy is nodular hyperplasia, with single or multiple nodules. The findings, however, vary considerably depending of the characteristics of the hyperplasia, the size of the nodules and their location, among other factors. Hyperplastic thyroid tissue has a mixture of follicles of variable size and morphology. They have a smooth surface, like a well-inflated beach ball (fig. 3, 4), but may show infolding. Depending where the core biopsy is taken, we may observe stromal fibrosis, past or ongoing haemorrhage, inflammation, interstitial oedema, presence of macrophages, cholesterol crystals, extravasation of colloid, reactions to foreign bodies and calcification. Specimens from central areas with sclerosis or scar tissue may resemble adenoma due to the preponderance of microfollicles. Hyperplastic cells are more elongated than normal cells but they still have small, hyperchromatic nuclei.

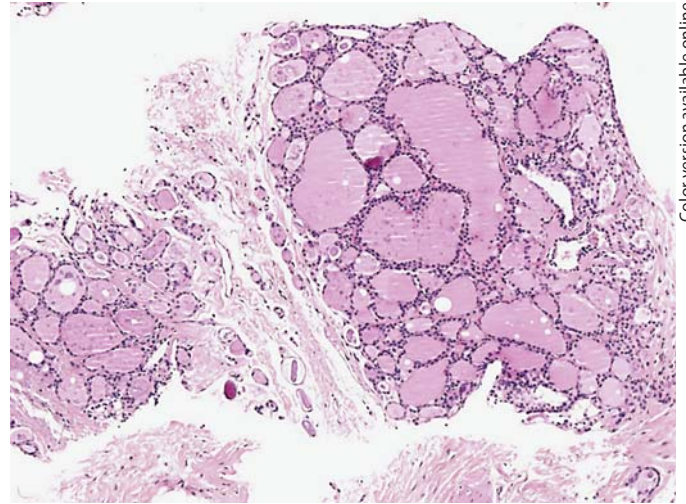
#### *Thyroiditis*

A clinical approach is essential to diagnose thyroiditis subtypes with the small amount of material available, since depending on the context the same histological im-



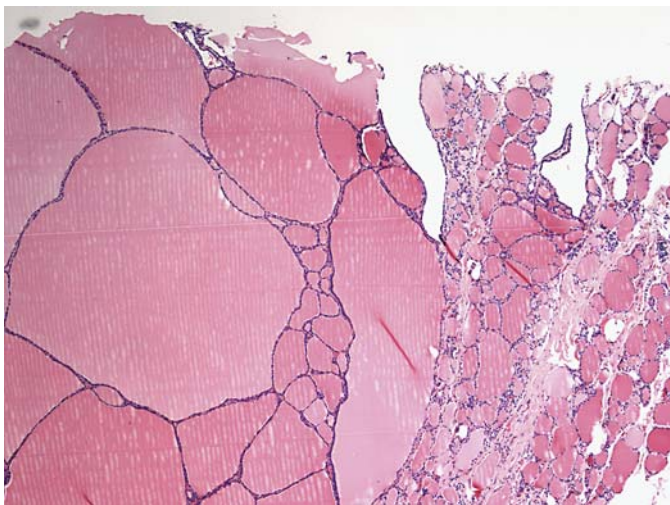
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**Fig. 2.** Normal thyroid tissue. Note the thyroid follicles with a non-smooth surface (like an underinflated beach ball) and thin stromal material, with oedema. HE.  $\times 100$ .



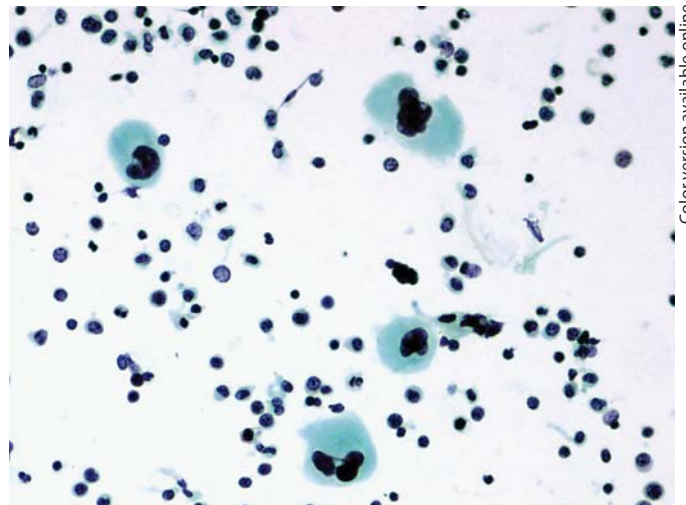
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**Fig. 3.** Hyperplastic thyroid tissue. The cylindrical specimens contain fibrotic bands separating groups of follicles of varied size and morphology. HE.  $\times 100$ .



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**Fig. 4.** Hyperplastic thyroid tissue. Note the thyroid follicles with a smooth surface (like an overinflated beach ball). HE.  $\times 100$ .

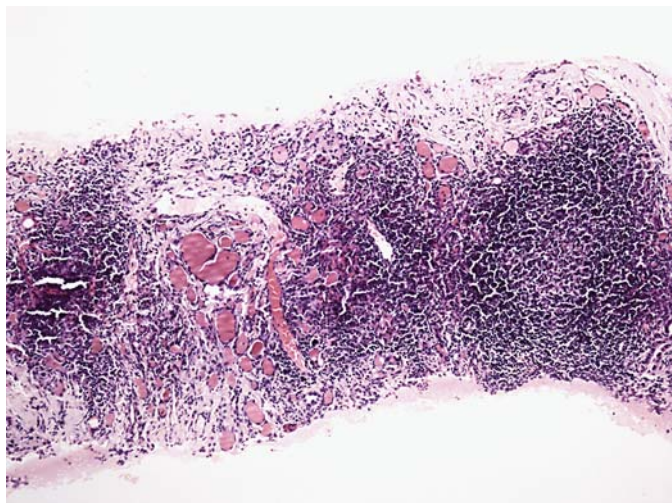


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**Fig. 5.** Hashimoto's thyroiditis. Cytological image of the cytocentrifuged transport medium. Note the loose epithelial cells with a large cytoplasm and oxyphilic features in an inflammatory background. Papanicolaou.  $\times 250$ .

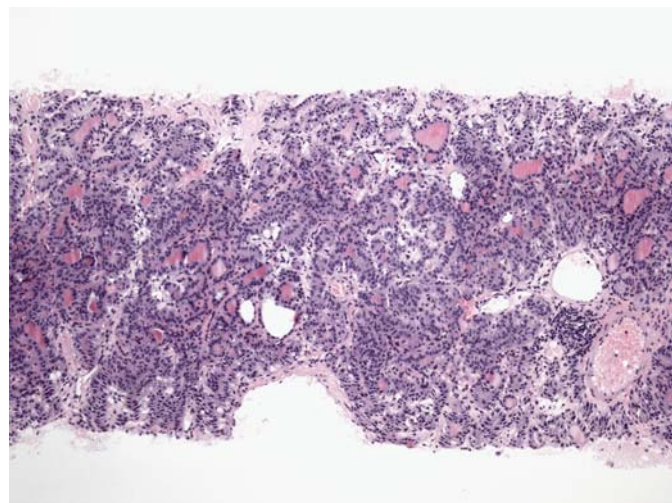
age may correspond to lymphocytic thyroiditis or to Hashimoto's thyroiditis, for instance. Removal of transport medium by centrifugation yields groups of follicular cells or individual cells in a lymphocytic background (fig. 5). Thyroid core biopsy specimens show significant lymphoid infiltrate (fig. 6), diffusely, in foci or even forming actual lymphoid follicles with clear centres.

With more severe inflammation, the number of thyroid follicles decreases and, at the same time, aggregates of microfollicles appear composed of thyroid cells with oxyphilic changes. A variable number of giant cells and plasma cells may be involved in the inflammation process. Depending on the course of the disease, there is a greater or lesser degree of stromal fibrosis. Notably, it can



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**Fig. 6.** Hashimoto's thyroiditis. Histological image of the cylindrical specimens showing the lobular architecture of the thyroid tissue, with fibrosis and patches of inflammation. HE.  $\times 40$ .



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**Fig. 7.** Papillary carcinoma. Panoramic image showing a proliferation of follicular structures in sclerotic stroma. HE.  $\times 100$ .

be very clear in the fibrosing variant of Hashimoto's thyroiditis and in the very rare condition Riedel's thyroiditis. Vigorous physical examination of the thyroid before the procedure increases the number of lymphocytes in the samples.

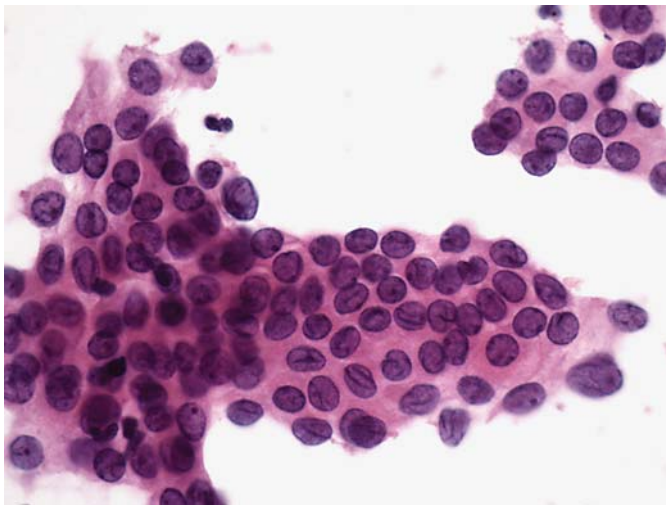
#### *Cysts and Other Lesions around the Thyroid*

An initial US scan is very useful to assess the nature of the cysts and to detect whether there are any solid areas, to then selectively sample these areas. Thyroid cysts may arise from embryonic remnants and, if so, they will develop along the thyroglossal duct (usually in the midline) or in a branchial cleft (laterally), or may be the result of secondary cyst formation from hyperplastic nodules. Generally, little or no solid tissue is obtained from cysts, while their content varies from colloidal to purulent. If we observe a transparent liquid, we should suspect that we have taken the sample from a cyst in the parathyroid gland. In the best-case scenario, we will obtain fibrous tissue from the cyst wall, generally without epithelium, containing some thyroid follicles. Depending on the exact location of the thyroid, its size and shape, and the type of disease involved, we may also sample lymph nodes, the thymus and parathyroid glands. It is common to find fragments of striated muscle tissue and/or adipose tissue together with thyroid tissue in core biopsy samples, while it is uncommon to find the normal fibrous capsule of the thyroid gland.

#### *Papillary Thyroid Carcinoma*

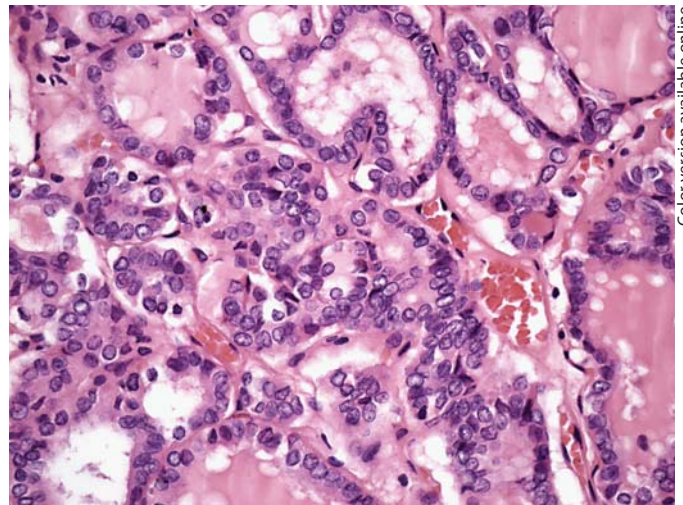
The diagnosis of papillary thyroid carcinoma is based on the characteristics of the nuclei. Given this, availability of only a small amount of material should not impede the diagnosis, as demonstrated in a recent study [18]. The main challenge, however, is sampling appropriately and knowing whether you have obtained representative samples. Specifically, as stated by LiVolsi and Baloch [19], papillary carcinoma may be an isolated tumour nodule, or be part of a hyperplastic nodule, either focal or multifocal. Given these characteristics, sampling is difficult, detection may be a matter of luck and usually very little tissue is obtained. It should be underlined, therefore, that it is possible for there to be papillary carcinoma close to a hyperplastic thyroid gland, and that diagnosis of one does not rule out the other.

There is a well-known association between Hashimoto's thyroiditis and papillary thyroid carcinoma [20]. In this case, the diagnosis of papillary carcinoma may also be difficult. In this type of cancer there may be papillae, which sometimes appear as foci but, on the other hand, there may not (fig. 7). Cytological analysis reveals cells with pale nuclei, sometimes overlapping and with grooves, forming papilla-like formations or poorly-cohesive clusters (fig. 8). In the histological analysis of core biopsies, the enlargement, overlapping and paleness of nuclei, together with nuclear grooves and pseudoinclusions are key diagnostic features (fig. 9). Sometimes psammoma bodies are also found. HBME-1 (fig. 10) and CK19 immuno-



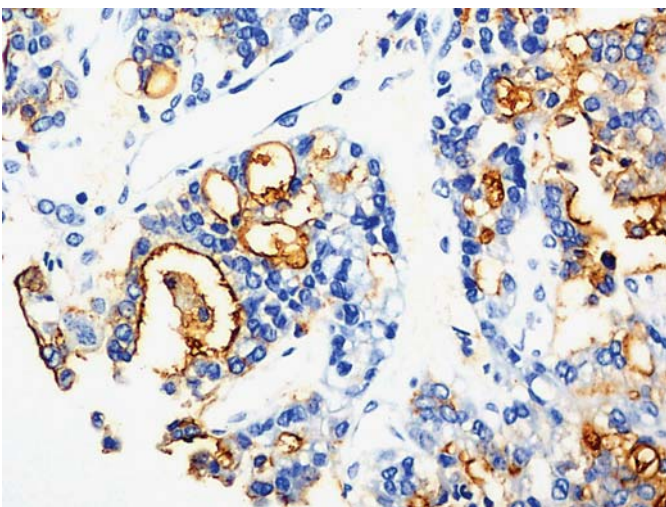
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**Fig. 8.** Papillary carcinoma. Cytological image of the transport medium showing cells containing pale, enlarged nuclei with grooves. Papanicolaou.  $\times 400$ .



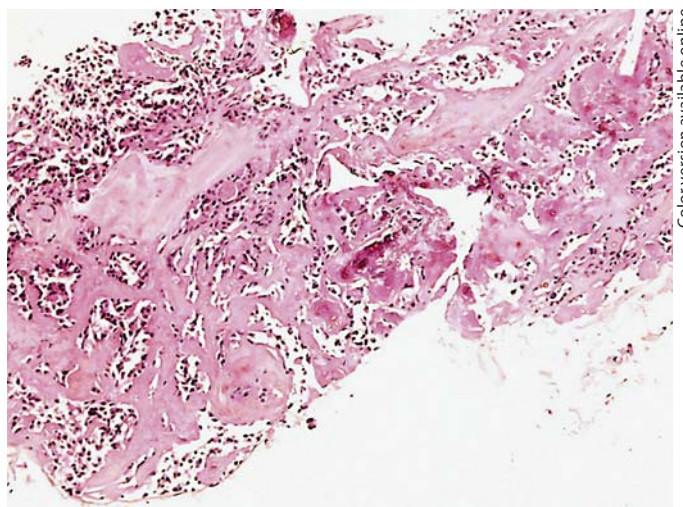
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**Fig. 9.** Papillary carcinoma. Histological detail showing the typical nuclear changes. HE.  $\times 400$ .



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**Fig. 10.** Papillary carcinoma. Strong positive staining with HBME-1. Dako, counterstaining with haematoxylin.  $\times 250$ .



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**Fig. 11.** Medullary carcinoma. Panoramic image showing stromal sclerosis with abundant eosinophilic material and cords of cells with a considerable amount of artefacts. HE.  $\times 40$ .

staining would help to identify whether these are papillary carcinoma cells. We should carefully assess the edges of the core biopsy samples, also evaluating extra-thyroidal areas, since in cases when tumours are found the data provided by the pathologist are of great clinical importance. In fact, it is well known that extension beyond the thyroid at diagnosis is one of the indicators of poorer prognosis in these patients.

### *Medullary Thyroid Carcinoma*

We should always suspect medullary thyroid carcinoma when the pathologist finds poorly-defined epithelial cell tumours. Diagnosis, based on haematoxylin and eosin (HE) staining, may be difficult with such small samples. However, a strong positive staining for calcitonin, chromogranin A and carcinoembryonic antigen, and a lack of staining with thyroglobulin clearly indicate the

nature of the cancer. Cytological analysis of the transport medium shows loose cells with oncocyte-like features using Papanicolaou stain. The tumour contains polyhedral cells forming solid lobes, nests, cords, and pseudoglands within a matrix with sclerosis (fig. 11), sometimes with deposits of eosinophilic and acellular material, identified as amyloid using specific techniques. Cells have a granular eosinophilic cytoplasm.

### *Lymphoma*

Primary lymphoma and plasmacytoma of the thyroid are rare, as is secondary involvement of the thyroid due to systemic metastasis. In this area, it is particularly important to view histological findings together with the clinical features of the patients. The presence of dense lymphoid infiltrate in the thyroid should make us suspect above all thyroiditis, the characteristics of which have been described above. Once thyroiditis has been ruled out, however, we must determine the nature of the lymphoid infiltrate considering the typical cytological and immunohistochemical features of lymphomas.

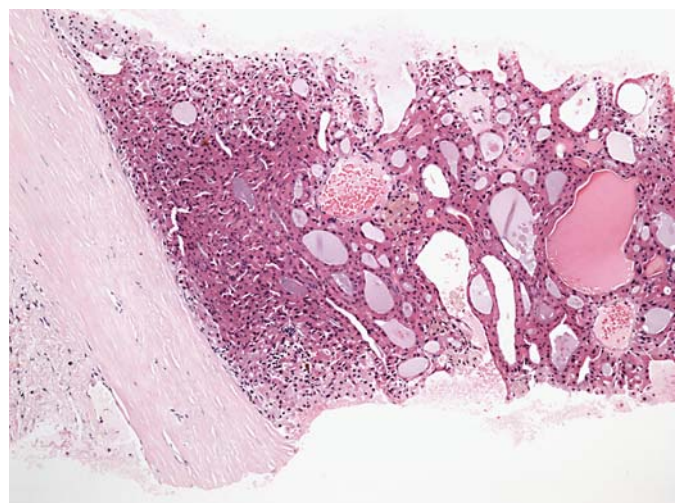
### *Other Types of Cancer*

Thyroid metastasis is uncommon. The primary types of cancer that most commonly metastasise to the thyroid are renal and lung carcinomas [21]. Somewhat more commonly, however, there is found to be secondary metastasis to the thyroid from other types of carcinoma in neighbouring organs and structures, such as the larynx and cervical lymph nodes. In all these cases, clinical features and radiological imaging of neighbouring structures should provide key information to support the histological diagnosis.

The following conditions, though very rare in the thyroid and unlikely to be found in core biopsies, could be suspected or even diagnosed if the defining histological characteristics were to be found in the specimens obtained: teratoma, ectopic thymic tissue, angiosarcoma, muscle-derived tumours, peripheral nerve sheath tumours, solitary fibrous tumours, Rosai-Dorfman disease, follicular dendritic cell tumours, and Langerhans cell histiocytosis.

### **Diseases That Cannot Be Diagnosed and Why Not**

We are not able to diagnose follicular adenoma/carcinoma in the usual form or their oncocytic variants (fig. 12). In this respect, the technique does not improve on the well-known limitations of FNA. In both cases, we



**Fig. 12.** Oncocytic tumour showing thick fibrous capsule and deeply eosinophilic cells arranged in follicles. HE.  $\times 40$ .

observe microfollicular proliferation without significant cytological findings, both after centrifugation of the transport medium and in histological slides. The pathological diagnosis of follicular thyroid carcinoma is a serious issue for patients since, although uncommon, it is very aggressive and has high metastatic potential. This type of cancer can only be diagnosed if there is unequivocal evidence of capsular and/or vascular invasion under the microscope [22]. These findings appear as foci in surgical specimens, and to make a diagnosis it is usually necessary to have sufficient tissue available to demonstrate this. Accordingly, there is almost no chance of obtaining definite histological findings consistent with this cancer in core biopsy samples, and hence its diagnosis is not possible.

### **Conclusions**

Obtaining thyroid tissue by US core biopsy is an inexpensive, rapid and reliable diagnostic procedure for thyroid disease. That said, FNAC remains a useful technique, and combining the two techniques does improve results, as demonstrated by several recently published series. The procedure to obtain and process core biopsy samples described in detail in this review provides histological slides and centrifuged transport medium, obtaining both at the same time. With appropriate material and relatively little experience, it is possible to reliably diag-

nose a very high percentage of cases, except follicular adenoma/carcinoma. The best results are obtained with the technique when the findings are reviewed by a multidisciplinary committee, to conduct a comprehensive analysis and reach a consensus on particularly difficult cases.

## Disclosure Statement

The authors have no conflicts of interest to disclose.

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