Chapter

Advances in Concepts, Ideas, and Methods Relevant to Fine Needle Aspiration Biopsy of Thyroid and Cervical Lymph Node

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Abstract

With the increasingly used semi-thyroidectomy and rapid progress in ultrasound-guided thermal ablation therapy for treatment of papillary thyroid carcinoma (PTC) and cervical lymph node metastasis from PTC, ultrasound-guided fine needle aspiration biopsy (FNAB) has got the mainstream position in pre-treatment cytopathologic diagnosis of PTC. How to acquire adequate and qualified cellular specimen for cytological examination has been described in several published expert consensus and practice guidelines. However, new issues continue to emerge in the real world of thyroid FNAB practice, and most of them are rooted in the perception and skills of the physician or technician who conduct FNAB. In this chapter, a series of new concept, idea, and technical methods are to be introduced and discussed. We believe that properly addressing these issues will facilitate the better implementation of FNAB and promote the new therapeutic modalities such as the thermal ablation to better progress.

Keywords: thyroid nodule, papillary thyroid carcinoma, cervical metastatic lymph node, parathyroid adenoma, benign sub-mandibular neoplasm, fine needle puncture, fine needle aspiration, fine needle injection, fine needle aspiration biopsy, fine needle aspiration cytopathology, needle channel vacuum aspiration, artificial vacuum aspiration, one puncture modality, 9 plus X-needle passages puncture modality, multiple puncture modality, liquid isolation method, liquid separation test, target shifting method, liquid dissection method, isolation liquid agent, saline, sodium hyaluronate gel, fine needle aspiration eluent, assay of thyroid hormone, assay of thyroglobulin, tumor infiltration adhesion, inflammatory adhesion, swallowing action, probe compress action, thermal ablation treatment, ultrasound guidance, systematic thinking and design

1. Introduction

Ultrasound-guided fine needle aspiration biopsy (FNAB) technology has been widely used for pathological diagnosis of thyroid nodule, and highly relevant expert

consensus or practice guidelines have been published at home and abroad [1–3]. It shows that its safety, accuracy, effectiveness, rapidity, and convenience have been widely recognized by many disciplines related to thyroid diseases and can be regarded as the mainstream method of preoperative pathological diagnosis of papillary thyroid carcinoma. However, the more extensive the clinical application it gets, the more likely to arise new problems it is. The more problems were promptly resolved, the better develop and improve can FNAB with times.

At present, the focus for not few operators in the implementation of FNAB is still on obtaining sufficient and high-quality cellular materials, preparing more standard cytological smears, and drawing exact cytopathological conclusions. There is no doubt that this is the goal of FNAB and must be perfect [4]. However, in face of the real situations of multiple thyroid nodules coexistence, metastatic lymph node coexistence, blood-rich nodules coexistence, and especially the situation of rapid popularity of thermal-ablation therapy for thyroid diseases, the authors consider it necessary and possible as well to further optimize the operation process of FNAB so as to explore its multi-purpose value and give play to other incidental functions while optimizing its main functions. In brief, we aim to make FNAB a versatile and unusual application technology.

In this chapter, the authors are to introduce their new original ideas, concepts, and methods relevant to thyroid gland and cervical metastatic lymph node and to share their clinical experience.

2. Concepts associated in the basic operation of fine needle aspiration biopsy

Fine needle aspiration (FNA) is not a fresh term in the field of interventional ultrasound medicine. As a pure modality of minimally invasive biopsy, it was developed nearly 30 years ago for achieving cytopathological diagnosis of liver neoplastic diseases. But it did not get widely used in clinic until the high-frequency ultrasound imaging techniques were increasingly used to examine thyroid glands and cervical lymph nodes. With the minimally invasive therapeutic concept gains popularity among the public, ultrasound-guided thermal ablation and hemithyroidectomy or even sub-lobectomy have been increasingly used to treat thyroid nodules or even papillary carcinomas. These new trends in surgical management of thyroid diseases have promoted the advance in concepts, ideas, and methods related to fine needle aspiration biopsy of thyroid and cervical lymph nodes.

2.1 FNP

FNP is the abbreviation of the phrase of fine needle puncture, which refers to a kind of super-minimally invasive operation by using a metal needle with an outer diameter less than 1 mm (called Chiba fine needle) to make percutaneous puncture into a target in the human body under real-time ultrasound guidance. Percutaneous puncture is the fundamental technic to perform interventional ultrasound medicine. Accordingly, FNP is the essential step for fine needle aspiration.

2.2 FNA

FNA is the abbreviation of the phrase of fine needle aspiration. To conduct FNA, one must perform FNP and operate the fine needle to aspirate the biomaterials from

the target lesion within the human body. Without FNP, FNA cannot be accomplished. Without aspiration, no biomaterials can be obtained.

2.3 FNAB and FNAC

FNAB is the abbreviation of the phrase of fine needle aspiration biopsy, while FNAC the phrase of fine needle aspiration cytopathology. In term of biopsy, there are two major ways. One way is incisional biopsy which used to be done by surgeon but has been seldom used nowadays. The other way is percutaneous puncture biopsy which recurs to handling a metallic needle under modern imaging guidance and has been increasingly used all over the world. FNAB is one of frequently used imaging-guided puncture biopsy. The goal of biopsy is to obtain adequate and qualified cellular specimen. From the term of FNAB, it can be concluded that the modality to implement biopsy is by FNA, not exfoliating cell examination or other methods. Only adequate and qualified cellular specimen has been available, can cytologists make effective microscopic examination of the specimen and accurate interpretation of the microscopic findings, i.e., the cytopathological diagnosis. The whole process from conducting FNA to ending FNAB and further from specimen processing to establishing cytopathological diagnosis is called fine needle aspiration cytopathology (FNAC). It is well known that FNAC is a frequently used modality to achieve fast cytopathology diagnosis of various thyroid diseases and cervical lymph adenopathy, especially the neoplastic diseases. Without the advances in FNAC, there would have been no progress in the minimally invasive thyroid surgery, including the ultrasound-guided thermal ablation treatment. FNA gets specimen through the action of aspiration. However, which force drives the aspiration? Universally, it is the native vacuum within the needle channel that generates the natural suction force to drive aspiration. However, under certain conditions, artificial suction force is needed to strengthen the vacuum for obtaining adequate specimen. FNP constitutes the base of FNA, but its roles are not limited to FNA.

2.4 FNI

FNI is the abbreviation of fine needle injection. It means to inject a liquid medium into a certain space in the human body through the fine needle channel. FNP is also the initial step for carrying out FNI, and FNI is another application of FNP. Compared with FNA, little concern has been given to the application of FNI. As a specific concept, FNI has not been raised ever before. However, this plain but important technic has been applied almost in every thermal ablation treatment for thyroid nodules, parathyroid adenomas, and cervical lymphatic metastasis, which is known as the liquid isolation method. With the progress of thermal ablation therapy in thyroid, parathyroid, cervical lymph node, and other neck organs, FNI has been given more missions. The missions of FNI include injection of anesthesia liquids, therapeutic liquids, and protective liquids into the peri-space of target nodule or target organ, to achieve analgesic, sclerotherapy or anti-infective therapy, and spatial separation as well.

Ultrasound-guided FNAB has been favored by multiple clinical disciplines such as the department of endocrinology, department of ultrasound medicine, department of head and neck surgery, and department of oncology. It has been playing a pivotal role in promoting the routine pathological diagnosis, special pathological diagnosis, and even genetic diagnosis of thyroid nodules. Unfortunately, in most cases, the work stays on conducting FNA only, and seldom has been done to FNI in the meantime.

Presently the surgical treatment mode of thyroid nodule is undergoing significant changes, that is, the treatment mode of preserving the native function of the gland to provide patients with long-term benefits is emerging gradually. Not only is the surgical resection method developing toward minimally invasive, but also the thermal ablation technology has become a new form of surgical treatment for benign nodules, hyperthyroidism, and even papillary thyroid carcinoma together with the metastatic cervical lymph nodes.

In this new situation, FNP of thyroid nodule should be done not only to meet the purpose of FNA but also to fully play the role of FNI in the mean time of FNA. With the help of action of aspiration (FNA) and injection (FNI), goals with integration of diagnosis and treatment can be readily achieved in the process of FNP of thyroid and cervical lymph nodes.

3. New concepts and methods associated in FNAB of thyroid nodules and cervical lymph nodes

3.1 Liquid isolation method

As a concept, liquid isolation method was created early in 2005 and was originally named as "hydro-dissection maneuver" by Zhang et al. [5]. It was designed purely as a protective measure for conducting a safe thermal ablation treatment of thyroid nodules under ultrasound guidance. With the expansion of thermal ablation modality to neoplastic diseases of parathyroid glands, sub-mandibular glands, parotid glands, and cervical lymph nodes, liquid isolation method has been applied almost in the whole neck area in Zhang's medical team [6]. The basic idea of this method is to inject some amount of saline into the peri-thyroid space to drive the adjacent structures move so much away as to insulate the heat radiation generated from microwave or radiofrequency conduction when thyroid nodule was in ablation (**Figure 1**). The area where the saline collects is called isolation zone. The shape of the zone is irregular. The width of the zone is variable. It gets narrowing as saline is absorbed and gets widening as more saline is injected (**Figure 2**). With the accumulation of clinical practice, we have found that the potential roles of liquid isolation method can be developed and optimized far beyond the role of protection from heat injury.

3.2 Isolation liquid agent

Isolation liquid means the liquid agent used for liquid isolation method. Saline or saline mixed with small amount of lidocaine is the most commonly used type of agent. Since its introduction into the procedure of thyroid thermal ablation for reducing post-ablation local adhesion in 2017 by Zhang et al. [7], sodium hyaluronate gel (SHG) was found to have the capability of forming an isolation zone which can provide more powerful driving force and last much longer time than saline isolation zone. Therefore, SHG has been increasingly used as new type of isolation liquid agent to provide safety for thermal ablation treatment of large thyroid nodule and/or multiple thyroid nodules which commonly needs much operation time.

3.3 Liquid separation test

Liquid separation test is derived from liquid isolation method with focus on the judgment of whether a thyroid lesion especially malignant tumor develops adhesion with the

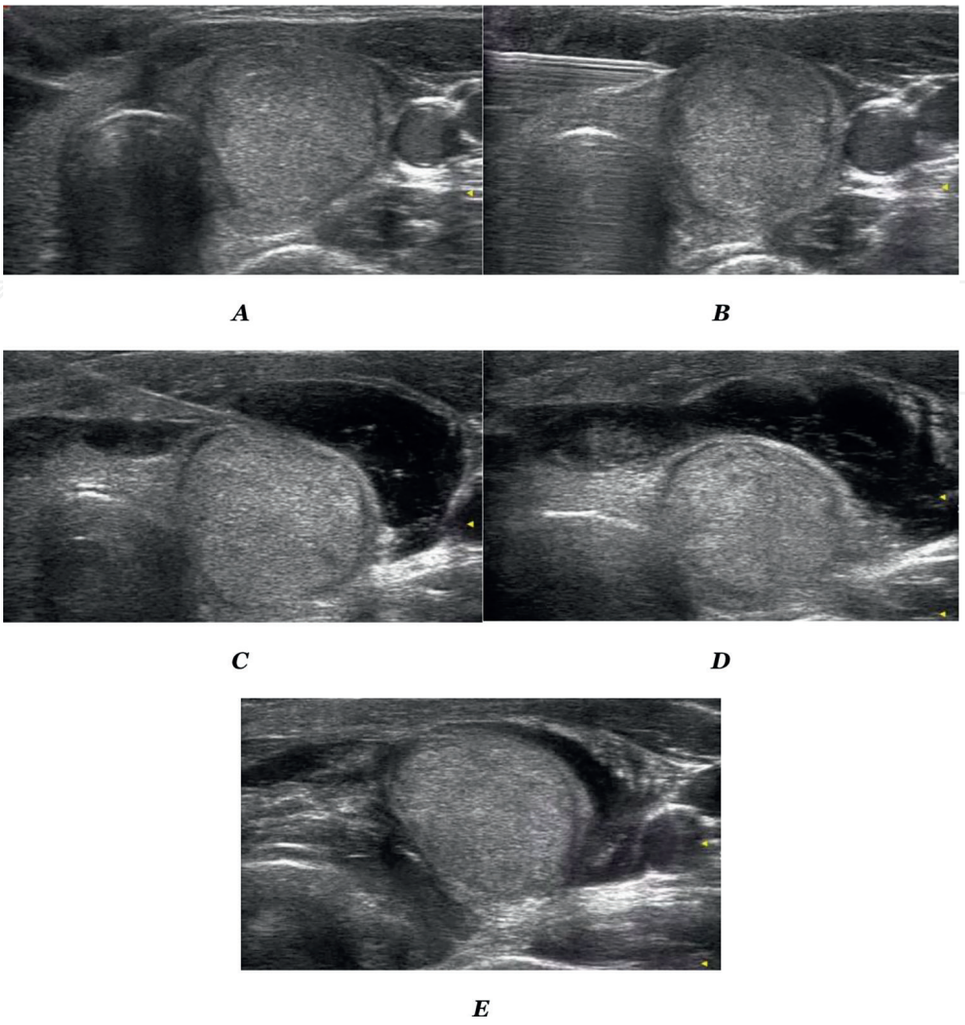


Figure 1.
Conduction of liquid isolation method under ultrasound guidance. (A) shows the natural state of a thyroid nodule in the right lobe, it was located closely adjacent to the trachea, right common carotid artery, strap muscle, and collilongus. (B) shows the introducing of fine needle and the tip of the needle reaches at the sternothyroid muscle. (C) shows an anechoic area of saline (isolation zone) appeared in the lateral space of thyroid. (D) shows the isolation zone branched out from the lateral interspace to the anterior interspace of thyroid. (E) shows the isolation zone developed in the medial interspace of thyroid to separate the thyroid nodule away from trachea.

closely adjacent structures. For doctors, to judge whether a thyroid lesion is indicated or contraindicated to thermal ablation treatment, it is essentially necessary to make it clear whether the lesion has been adhesive to adjacent structure especially the trachea, esophagus, recurrent laryngeal nerve, or vital blood vessels (**Figure 3**). If adhesion is presented, thermal ablation treatment is strictly forbidden to be used. As grows the demands for thermal ablation therapy by the patients with papillary thyroid carcinoma (PTC) or with cervical lymph node metastasis post-surgical resection of PTC, liquid separation test has been increasingly performed in the meanwhile of FNAB. The practice under this new concept has facilitated the doctors to promptly learn the real state of patient's condition and make appropriate treatment protocol with saving consultation time.

3.4 Target shifting method

Target shifting method means an artificial method to make the target lesion in thyroid or other organs move to intended location fit to FNAB or thermal ablation by the

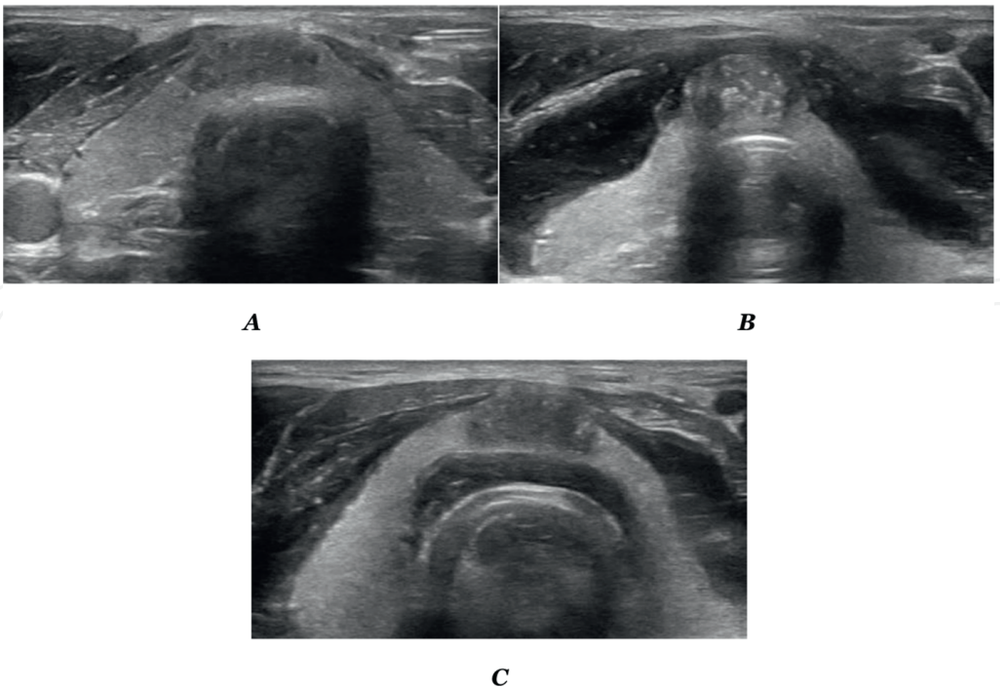


Figure 2.
Dynamic changes in the shape and width of isolation zone. (A) shows the natural state of a thyroid nodule at the isthmus, it was located closely adjacent to the sternothyroid muscle and the trachea. (B) shows that the isolation zone was formed in the anterior interspace of the thyroid but the width of the zone rightly before the isthmus was markedly smaller than what before the bilateral lobes. (C) shows that the isolation zone was formed in the posterior interspace of the isthmus but the anterior isolation zone got narrowed due to the absorption of saline.

driving force of the liquid injected. With the assistance of this method, the puncture route can be optimized with the target lesion moving to the appropriate puncture route or the neighboring structures moving away to open an appropriate puncture route (**Figure 4**). Meanwhile the display quality of the target lesion can be simultaneously improved (**Figure 5**).

3.5 Liquid dissection method

Liquid dissection method is also derived from liquid isolation method. It focuses on how to highlight the display of cervical metastatic lymph nodes, normal parathyroid glands, nerves, and sympathetic ganglion. Like surgical dissection to make clear exposure of target lesion in surgery, in the process of ultrasound-guided thermal ablation the target lesion needs clear display as well, and otherwise the operator cannot make definite location of the target and cannot treat the lesion safely and efficiently. However, the surgical tools and surgical dissection method cannot be directly used in this situation. Liquid driving force is the only available to make the target separated from the surroundings and come to the best display on ultrasound imaging (**Figure 6**). This process is so called as liquid dissection.

3.6 FNAB immediately after heat blocking nutrient arteries

The core idea of this method lies in blocking the blood supply to the target lesion with microwave or radiofrequency energy rightly before doing FNAB. Thyroid

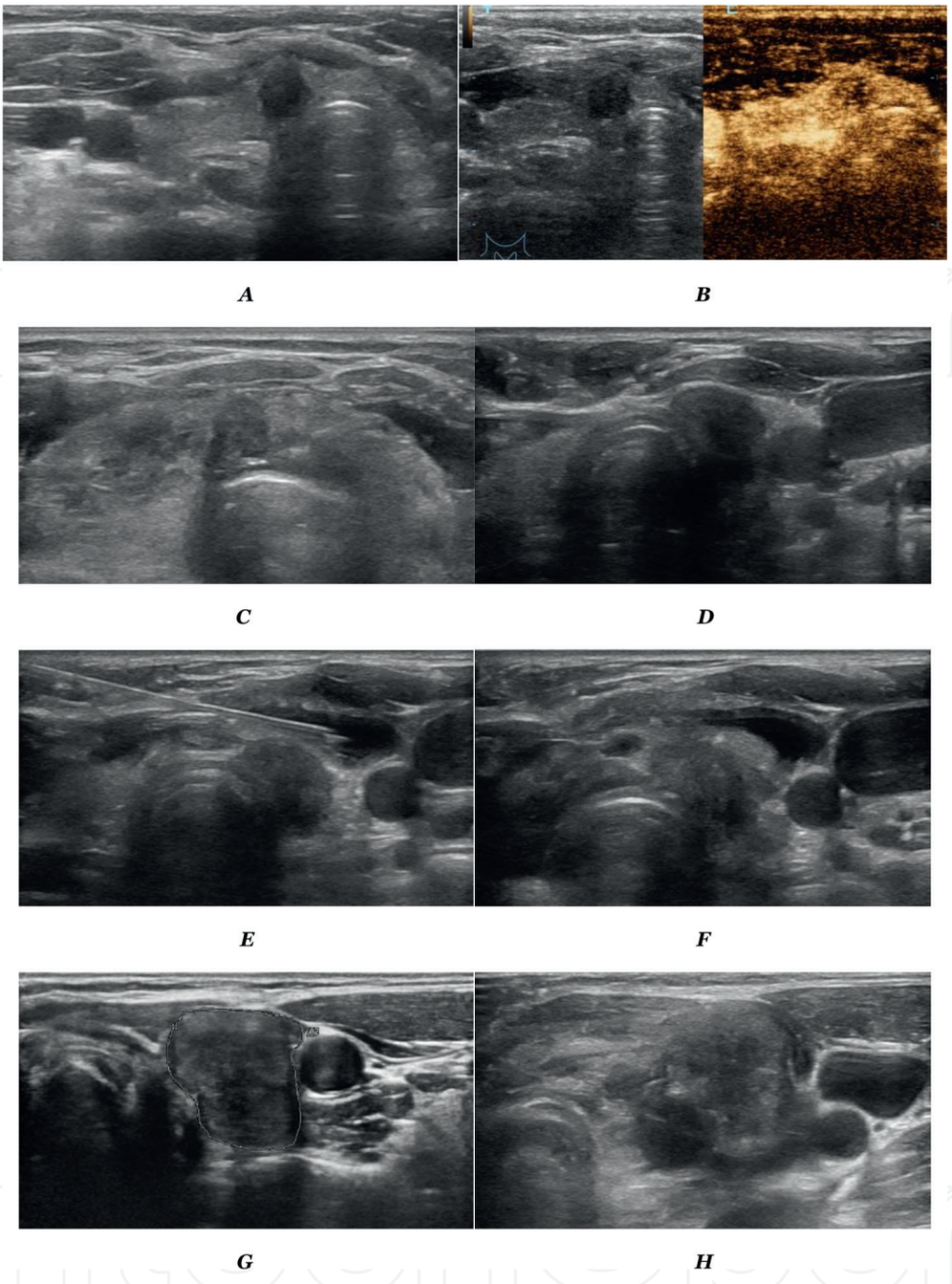


Figure 3.
Identification of adhesion between thyroid nodule and adjacent structure by liquid separation test. (A) shows that a hypoechoic nodule was located at the isthmus and closely adjacent to the trachea but the adhesion of the nodule with trachea was beyond confirmation. (B) shows the microvasculature blood perfusion of the nodule on contrast-enhanced ultrasonography of the nodule but no valuable information was obtained to diagnose the adhesion. (C) shows the isthmus nodule failed to be separated from the trachea and no isolation zone developed in the posterior interspace behind the nodule while saline was injected. (D) shows that a hypoechoic nodule was located at the lower pole of right thyroid lobe and closely adjacent to the right lateral wall of the trachea with high suspicion of adhesion to the trachea. (E) shows that isolation zone was formed in the anterior interspace of the thyroid indicating absence of adhesion between the nodule and strap muscle. (F) shows that isolation zone failed to form in the medial interspace with failure of separation of the nodule away from the trachea indicating the presence of tight adhesion. This nodule was not believed to meet the indication for thermal ablation therapy. (G) shows that a relatively large hypoechoic nodule was located at the lower pole of right thyroid lobe which had close contact both to the trachea and common carotid artery. (H) shows that isolation zone was formed in the media and lateral interspace of thyroid indicating the separation of the nodule away from the trachea and common carotid artery. This nodule was believed to meet the indication for thermal ablation therapy.

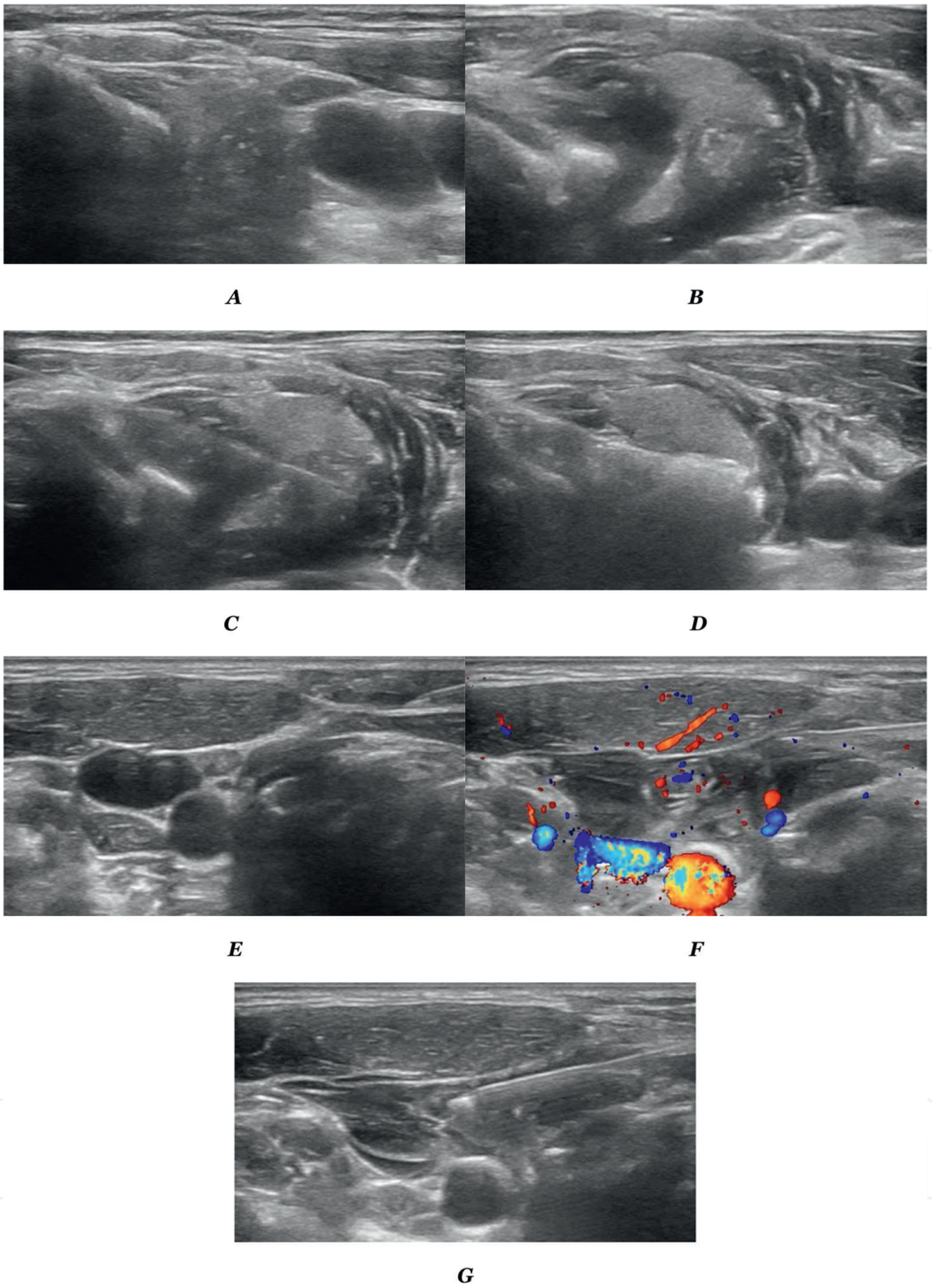


Figure 4.
Optimizing the puncture route by targeting shifting method. (A) shows a hypoechoic nodule with fuzzy boundary was located at the upper pole of right thyroid lobe and was closely adjacent to the thyroid cartilage and common carotid artery. It was not believed safe and convenient to perform either FNAB or thermal ablation in its plain state. (B) shows the nodule was shifted to the new position under the driving force of saline injection. (C) and (D) show respectively the nodule was appropriate for safe and convenient FNAB and thermal ablation treatment after shifting to the new position. (E) shows a small cervical lymph node suspicious of metastasis in Region VI was located closely adjacent to the trachea, common carotid artery and internal jugular vein. (F) shows after injection of some amount of saline the common carotid artery and internal jugular vein moved away far from the lymph node and the lymph node moved away from the trachea. (G) shows with the adjacent vital structures shifting somewhere the implementation of FNAB was under safe guaranty.

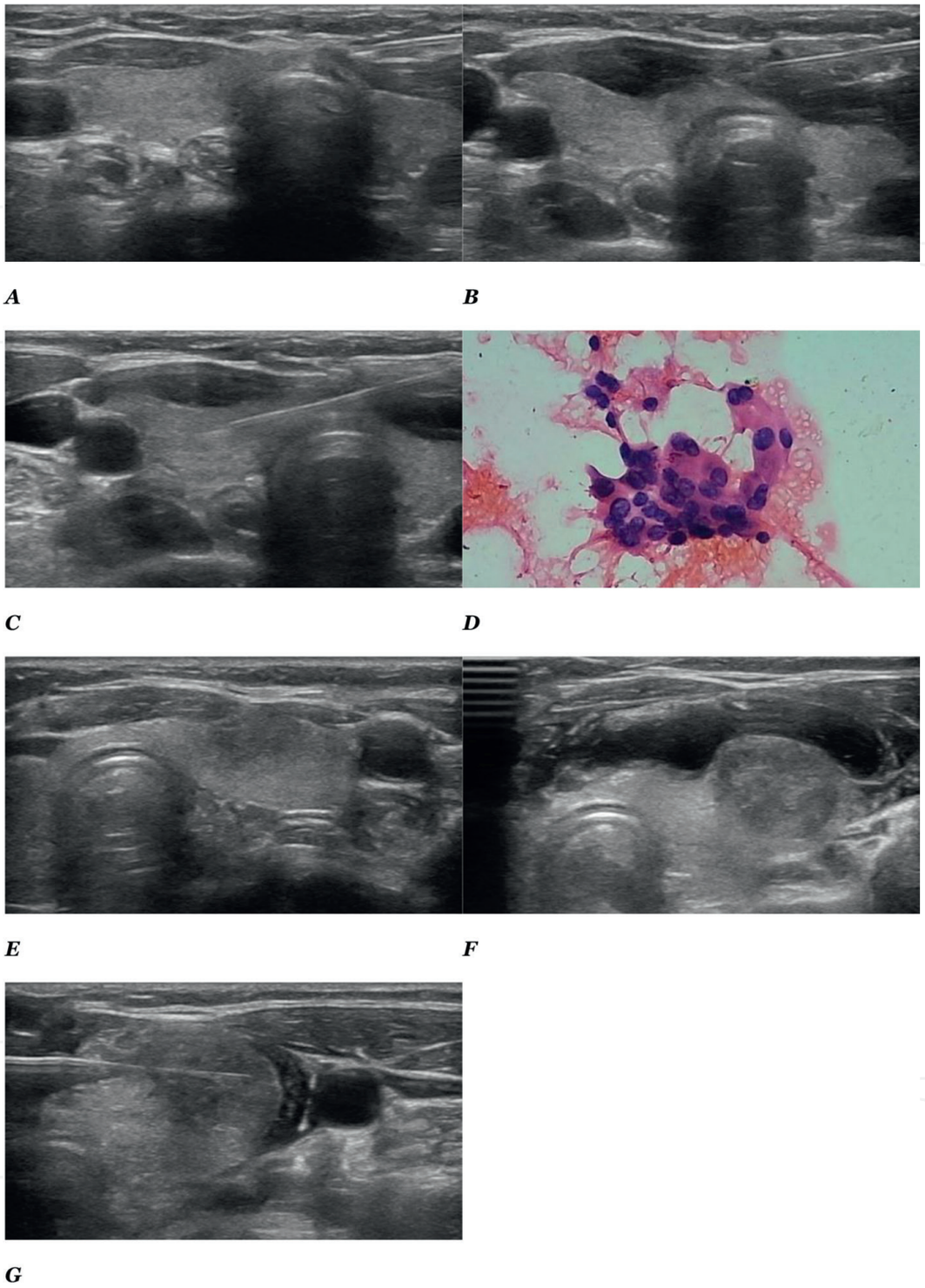
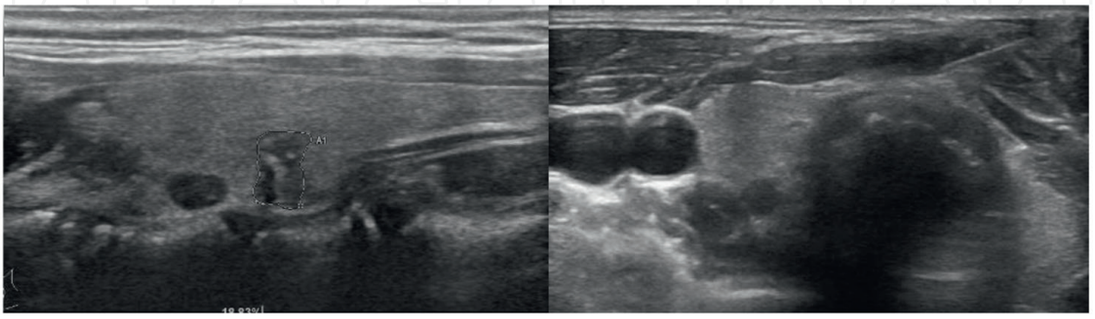


Figure 5.
Optimizing the ultrasonic display of tiny thyroid nodule by liquid shifting method. (A) shows no definite nodule on the section of thyroid. (B) shows a tiny hypoechoic nodule appeared at the joint part of isthmus and left lobe of thyroid with the aid of injection of saline to form the isolation zone in the anterior interspace compared to (A), indicating liquid isolation zone significantly improved the ultrasonic display of target lesion. (C) shows the process of FNAB of the tiny nodule. (D) shows the cellular appearance of the specimen obtained from the tiny nodule, which highly indicated the presence of papillary thyroid carcinoma. (E) shows that a moderately hypoechoic nodule with unclear boundary was located at the lower pole of right thyroid lobe. (F) shows that the nodule turned clear in boundary with the injection of saline to the anterior interspace of thyroid. (G) shows that the process of FNAB and cytopathologist confirmed the diagnosis of this nodule as PTC.

nodules rich in blood supply are not uncommon. The color Doppler flow imaging (CDFI) mode is sensitive in detecting the blood flow associated with the thyroid nodules and presenting them as fruitful color signals. FNAB on such nodules, if without any preparation for processing, can inevitably induce active bleeding within the thyroid gland or even among the peri-thyroid space. Moderate to severe bleeding would bring the patient a swollen neck or even difficult breathing, which needs an intensive care. For the part of such cases, it is most appropriate to implement FNAB



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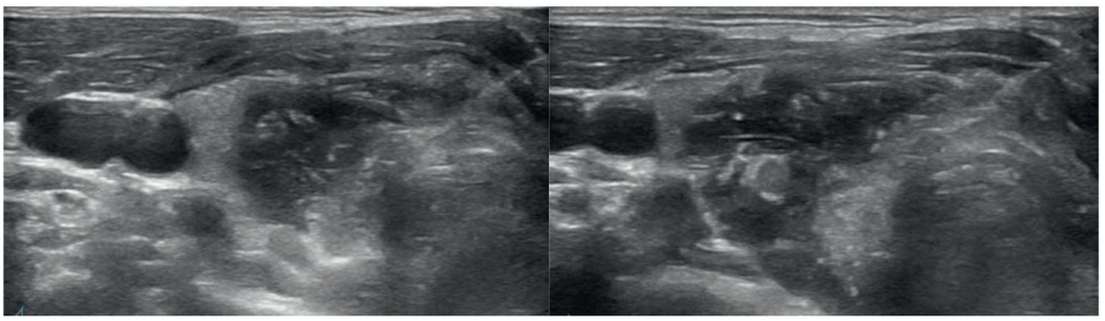

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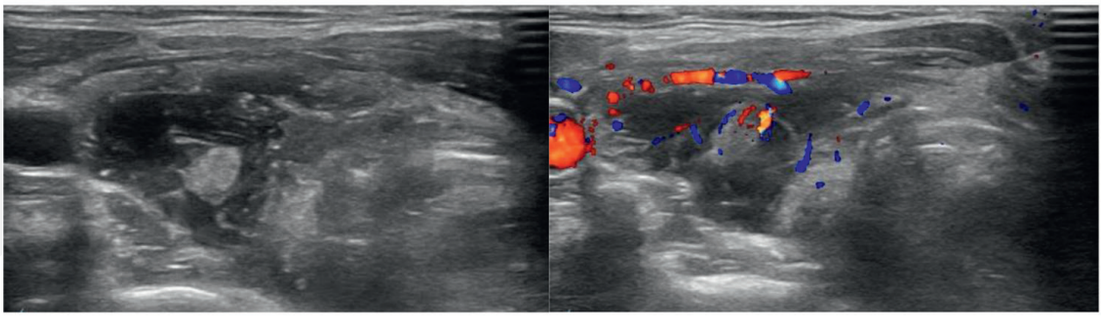

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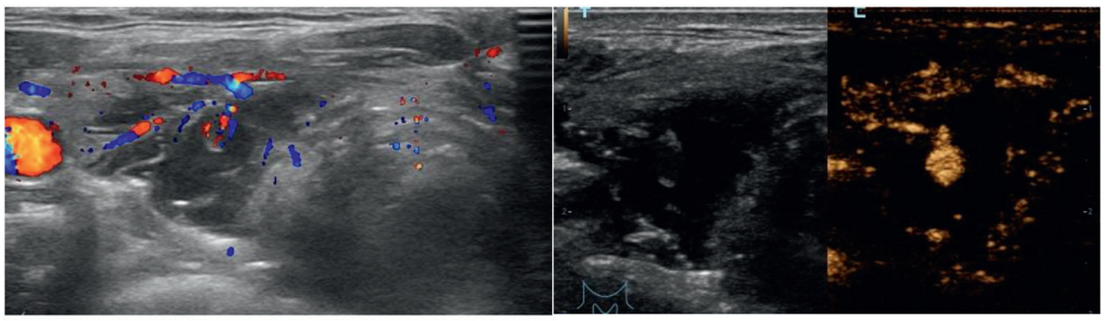

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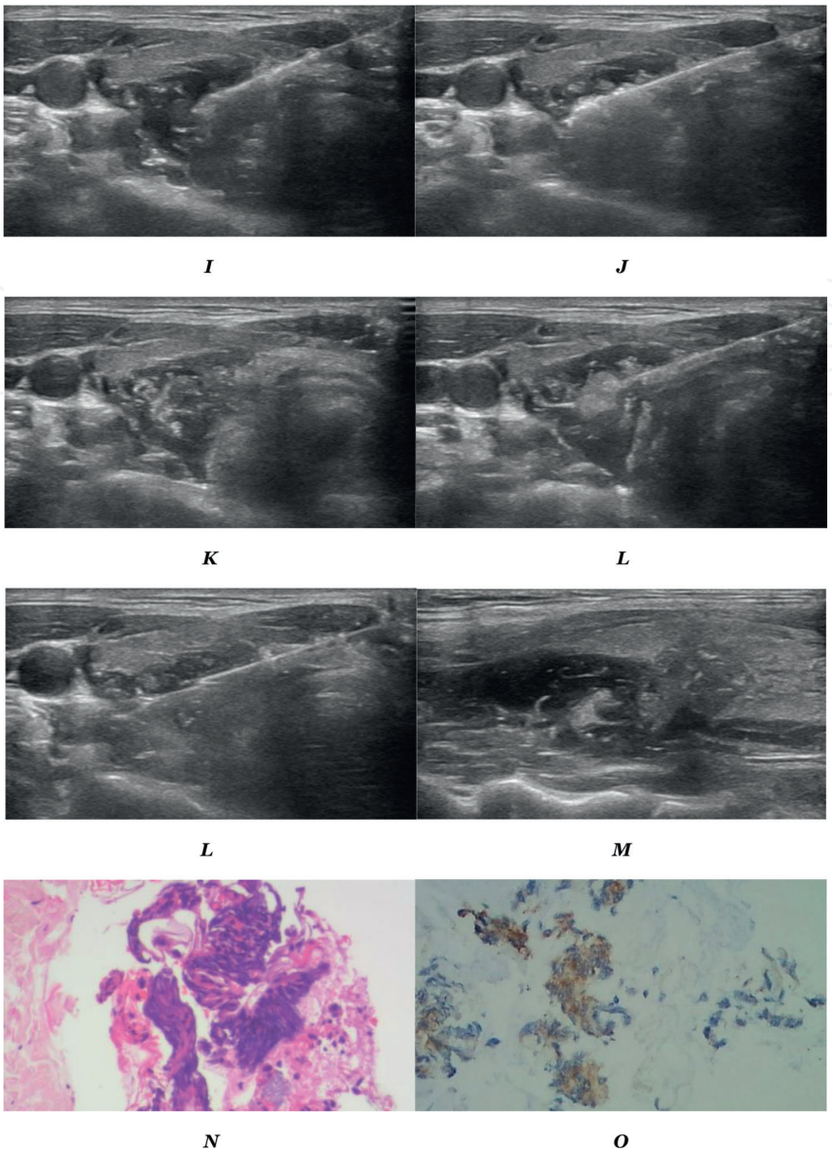


Figure 6.
Identifying and conducting FNAB of parathyroid lesion by liquid dissection method. (A) shows that two small hypoechoic nodules were located beneath the posterior capsule at the middle portion of the left thyroid lobe with one in homogenously hypoecho and the other in heterogeneously hypoecho with multiple calcifications. (B) shows the nodule in homogenously hypoecho on transverse section ready for administration of local anesthesia for FNAB. (C) shows that the nodule was separated from the left thyroid lobe, indicating the nodule was not originated from thyroid. (D) shows that the nodule was totally surrounded by the saline and free to the left thyroid, esophagus, and trachea, indicating a lesion arising from superior parathyroid gland. (E) shows a moderately and homogenously hyperechoic nodule in oval shape closely beside the hypoechoic nodule shown in D, indicating as a part of normal parathyroid tissue. (F) shows the fine arterial vessels supplying the normal part of the parathyroid gland on CDFI scanning. (G) shows the fine arterial vessels supplying the lesion of the parathyroid gland on CDFI scanning. (H) shows the homogenous hyperenhancement within the microvasculature of the parathyroid lesion on contrast-enhanced ultrasonography. (I) shows that microwave antenna was inserted to the hypoechoic area of parathyroid lesion. (J) shows that the hypoechoic parathyroid lesion turned extensively hyperechoic immediately after microwave energy was output. (K) shows that the hypoechoic lesion of parathyroid gland had changed in size, shape, and echogenicity after MWA, indicating the diseased part of the parathyroid got well treated with heat coagulation. (L) shows the previously normal part of parathyroid stayed almost unchanged in size, shape, and echoes after MWA, indicating the normal part of the parathyroid gland got well protected from the heat injury. (M) shows that a core needle biopsy (CNB) was conducted with a 16G core-cutting needle on the thermally ablated lesion for reaching a histopathological diagnosis. (N) shows the entire left superior parathyroid gland on longitudinal section, indicating that the normal part was still normal and the diseased part was treated as well. There was no active bleeding visible on the ultrasonographic examination. It is true that the discovery, identification, microwave ablation, and core needle biopsy of such tiny incidentaloma of parathyroid gland in this case would have not been possible if without the help of liquid dissection method.

together with the thermal ablation treatment of the nodules in view of safety. With color Doppler scanning, the nutrient arteries to the target nodule are clearly and thoroughly identified. While MWA or RFA was started, the ablation antenna or electrode was first inserted to the site of color signals of arteries closely prior to nodule to coagulate them until repeated CDFI scanning showed the blood signals related to the nodule had thoroughly disappeared (**Figure 7**). Then, FNAB of the nodule was implemented immediately. As the ablation needle was set outside of the nodule, so the tissue within the nodule was free of coagulation. By this method, the risk of severe bleeding secondary to FNAB was significantly reduced, but the cellular specimen obtained remained in natural state without heat injury [8] (**Figure 8**).

3.7 FNAB immediately after heat coagulation of nodule tissue

This method is derived from the method of FNAB immediately after heat blocking nutrient arteries. It is not uncommon that follicular thyroid nodules are rich in colloid contents and blood supply as well. FNAB on such nodules would not only cause active bleeding but also extract unqualified cellular specimens. If the specimen contains more colloid and blood but less follicular cells, it gets hard for cytologist to make an accurate assessment. Such cellular specimen is believed unqualified. However, heat coagulation can close the blood vessels to reduce the risk of bleeding and evaporate water to reduce colloid with a joint result of improving the quality of biopsy specimen. For the part of such cases, one can coagulate the entire nodule first and conducts FNAB afterwards. Due to the intensive action of heat, part of the cells in the biopsy specimen would present spindled morphology under microscopy (**Figure 9**), but if the cytologist had been informed how the specimen was taken off, the correct cytopathological diagnosis would be reached all the same.

3.8 "9 + X"-needle passages puncture modality

This concept was raised first by Zhang et al. in 2019 [9]. In the definition of this puncture modality, the target nodule is divided into three areas, that is the superior, the middle, and the inferior area on the longitudinal section with ultrasound imaging. Each area is subsequently subdivided into another three small parts, that is the anterior, the middle, and the posterior part on the transverse section. Thus, the target nodule can be divided into nine partitions as needed (**Figure 10**). While performing FNA, the operator successively introduces the fine needle into the nine partitions to collect cellular components from the nine needle passages before withdrawing the fine needle out of the body. By the modality of nine needle passages puncture, the fine needle needs only one entry and exit of the patient's body to obtain sufficient cell samples from nine partitions of the target lesion. A complete FNAB operation means that the fine needle enters and leaves the patient's body only once, no matter how many times the needle aspirates within the target lesion. This is called one needle puncture skill. Through this skill specimen of biopsy can be obtained adequately with less mechanical damage to patient's body. The nine needle passages puncture modality fits the feature of one-puncture operation. Although there are nine needle passages within the target lesion, there is only one subcutaneous needle passage from the skin to the thyroid capsule.

Here, X refers to the uncertain number of extra needle passage. If there are special ultrasonic features presenting in areas of the target lesion such as microcalcification, extremely rich of or lack of blood flow signal, or relatively high elastic strain ratio, an extra

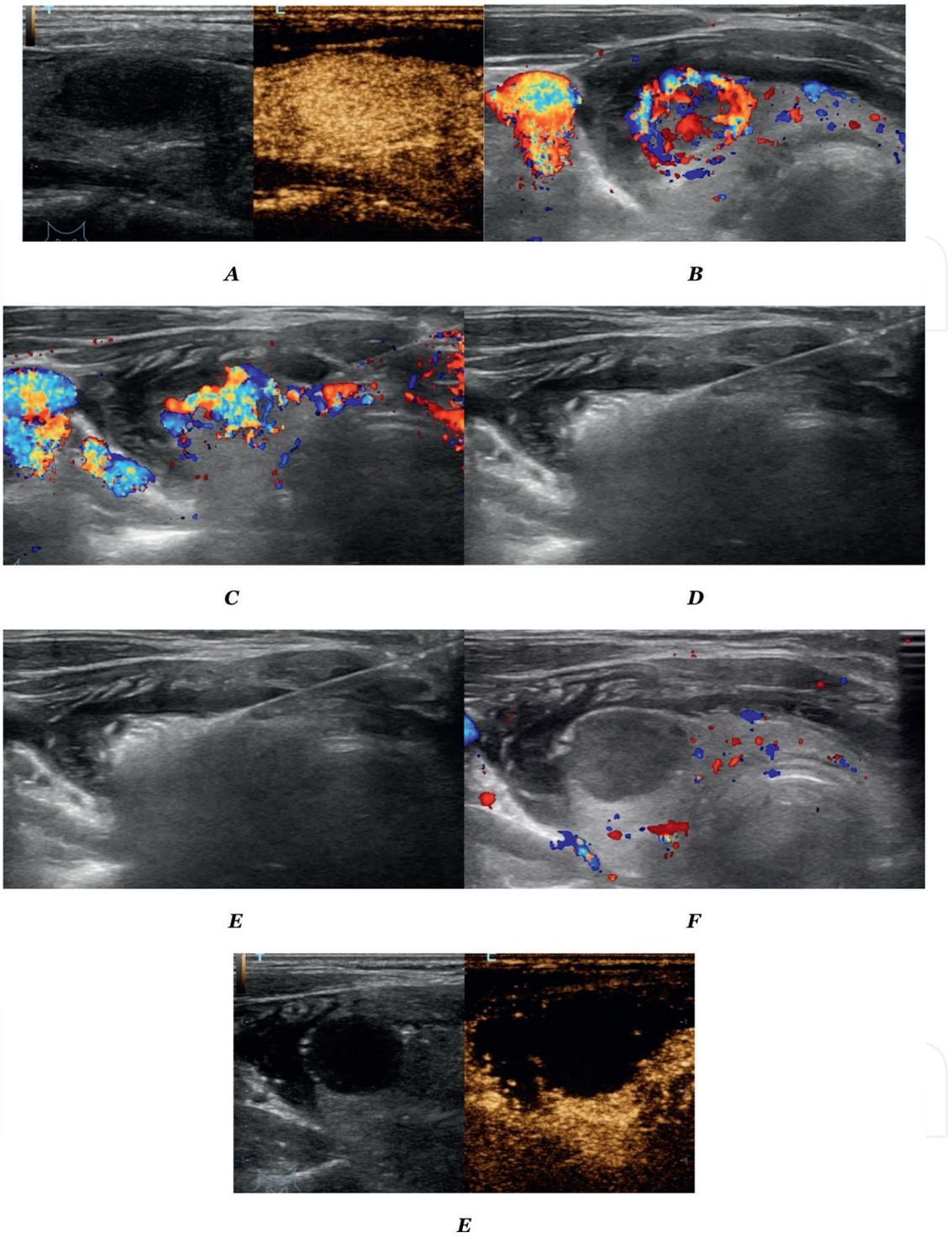


Figure 7.
Method and flow for conducting FNAB immediately after heat blocking nutrient arteries. (A) shows that a homogenously hypoechoic thyroid nodule was in entire hyperenhancement on CEUS scanning. (B) shows that the thyroid nodule was filled with a wheel-like color signal on CDFI scanning, indicating the high risk of bleeding associated with FNAB. (C) shows that there was a mess of brightly mosaic-like color signal closely prior to the thyroid nodule, which was the major arterial branch to nutrient the nodule. (D) shows that a microwave antenna was inserted into the area of mosaic-like color signal and the area appeared extensively hyperechoic, indicating that the microwave ablation was undergoing. (E) shows the original color signal covering the entire thyroid nodule disappeared thoroughly immediately after heat coagulation of the supplying vessels, indicating a successful block of nutrient artery. (F) shows the nodule turned entirely filling-defected on CEUS scanning, furtherly confirming the successful complete block of nutrient artery.

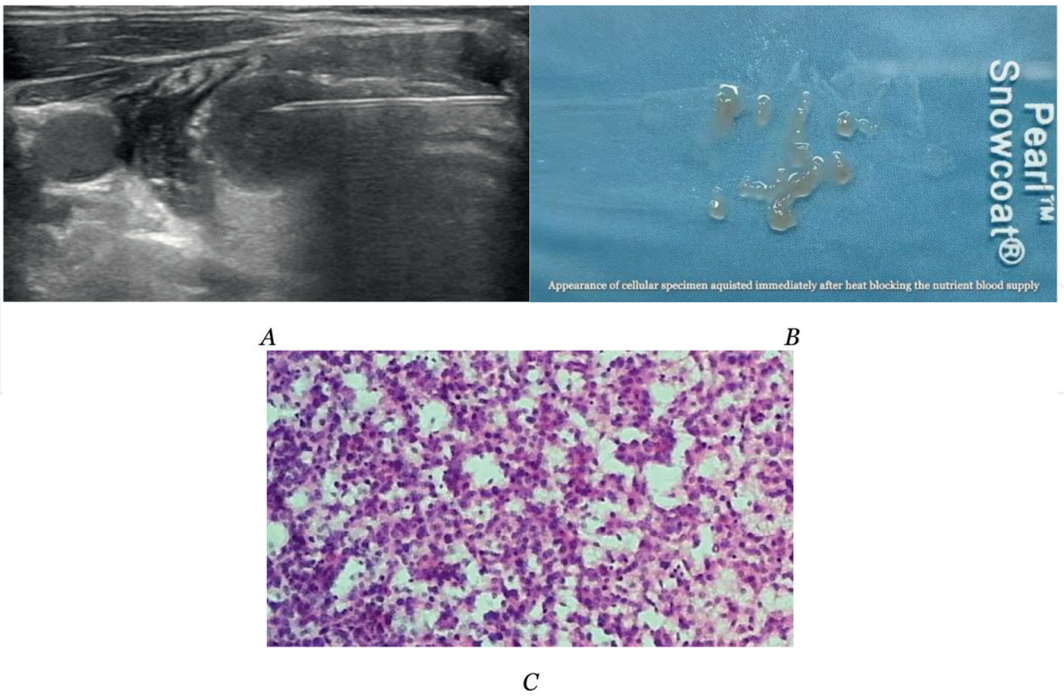


Figure 8.
Comprehensive figures of thyroid nodule immediately after heat blocking nutrient arteries. (A) shows that FNAB was conducted immediately after the nutrient artery was blocked. (B) shows the fresh appearance of cellular specimen from the nodule in colloid-like and no blood component. (C) shows the microscopic findings of the specimen that the thyroid epithelial cells were in uniform distribution and arrangement, with small follicles but not any atypia, indicating the diagnosis of benign thyroid nodule.

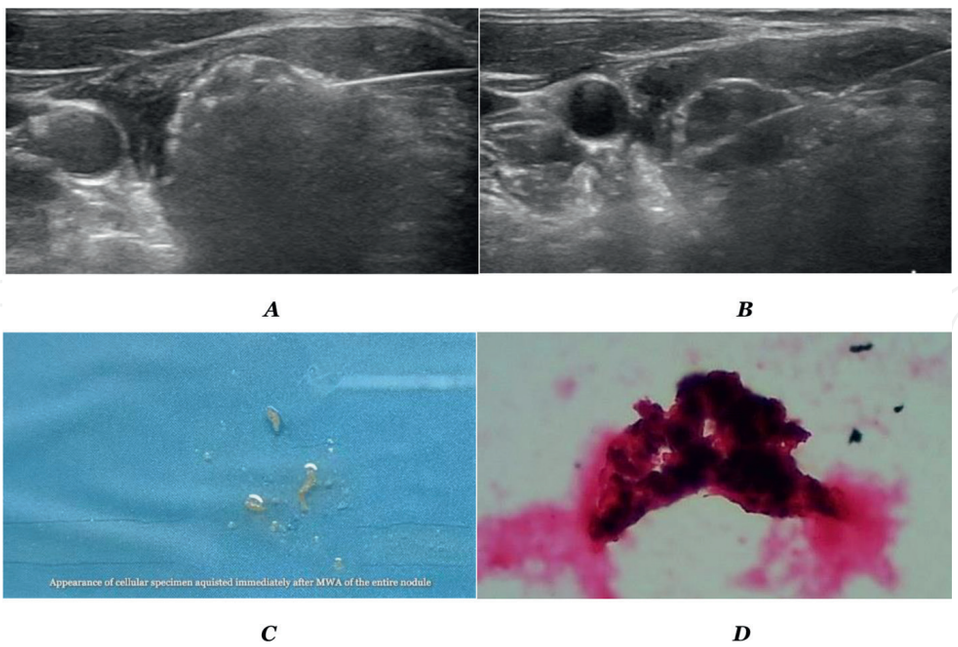


Figure 9.
Method and flow for conducting FNAB immediately after coagulation of nodule tissue. (A) shows that microwave ablation was conducted on the entire nodule, indicating the cells in nodular tissue suffering heat damage. (B) shows that FNAB was performed immediately after the entire nodule was ablated. (C) shows the fresh appearance of cellular specimen from the ablated nodule, indicating quite difference from what after blocking nutrient artery. (D) shows the microscopic findings of the specimen from the nodule that a small clump of thyroid epithelial cells were agglutinated, unlike the uniform distribution and arrangement before ablation, indicating the cells suffering heat damage.

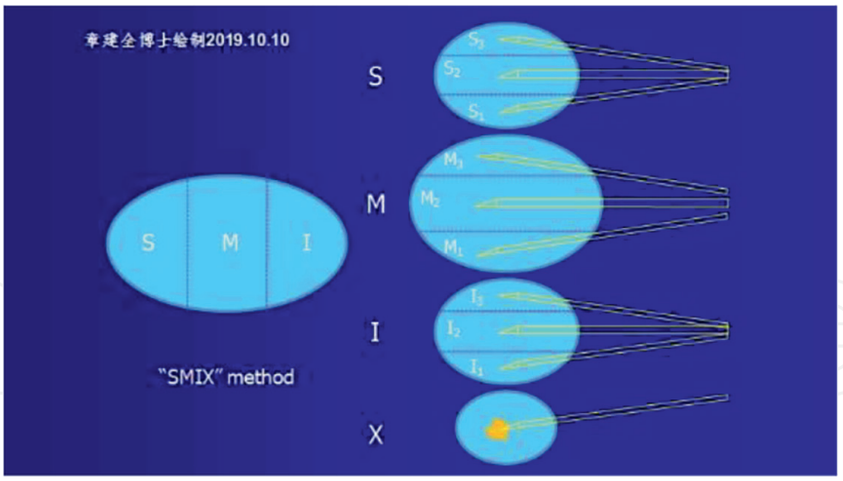
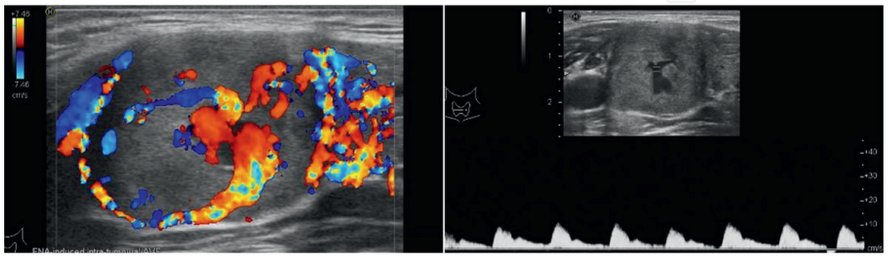


Figure 10.
Schematic diagram of "9 + X"-needle passages (or SMIX) puncture modality.

FANB sampling at the site of special feature is subsequently conducted for additional smear slice. This is the concept of 9 + X needle passage puncture mode. As the cellular specimen comes from the superior(S), middle(M), inferior(I) partitions, and uncertain foci(X) of the nodule lesion, 9 + X needle passage puncture mode can be called as SMIX puncture mode alternatively.

The advantage of adoption of "9 + X needle passage puncture mode" is to take into consideration the comprehensiveness of the sampling sites as much as possible, the adequacy of the sampling quantity, the optimization of the sampling quality, and the evidence of the ultrasonographic manifestations. It is the high efficiency, sufficient quantity, high quality and minimal injury that the progressiveness of "9 + X"-needle passages puncture modality lies in. It is a beneficial innovative exploration for improving the standardization and rigor of thyroid nodule FNAB.

The counterpart of one-puncture technique is multi-puncture technique. Multi-puncture technique means that the puncture needle enters and exits patient's body commonly 3 to 4 times. Although this method can also increase the total amount of specimens, it is obvious that multi-puncture will increase the mechanical injury of the subcutaneous tissue, thyroid capsule, thyroid parenchyma, and easily increase the risk of bleeding or even the formation of pseudoaneurysm. As illustrated in **Figure 11**, a 24-year-old girl underwent the procedure of FNAB of her right thyroid nodule and FNAC revealed the cellular feature of benign tumor. Unexpectedly, one week after FNAB. A painful



A ***B***

Figure 11.
Multimode ultrasonographic features of intrathyroidal pseudoaneurysm induced by FNAB. (A) shows that an echo-free area within the thyroid nodule was filled with bright mosaic color signal on CDFI scanning. (B) shows that pulsed-wave Doppler detection revealed arterial flow profiles of the mosaic color signal, indicating the presence of pseudoaneurysm.

enlargement appeared at the site of her right thyroid. Follow-up ultrasound examination discovered that an echo-free area was formed within the thyroid nodule, which was not present at time of the FNAB. CDFI scanning revealed bright mosaic color signals filled the echo-free area in a pulsed rhythm. Pulsed-wave Doppler ultrasound detection presented the arterial flow profiles of the color signals. According to findings on CDFI and PW integrated with the history of FNAB, the diagnosis of FNAB-inducing intrathyroidal pseudoaneurysm was established. Afterwards, the pseudoaneurysm together with the thyroid nodule was successfully treated by using microwave ablation in our team.

For a qualified FNAB, it must be confirmed on the spot that the quantity of specimens is sufficient and up to cytopathological standard to avoid the second puncture operation caused by insufficiency of specimens. In particular, the "9 + X needle passage" puncture mode can not only obtain more comprehensive and adequate specimens, but also reduce the damage caused by multiple needle puncture. Therefore, it can be used as the first choice for large thyroid nodules.

4. New perception on issues involved in fine needle aspiration biopsy of thyroid nodule

4.1 Advocating comprehensive biopsy of multiple thyroid nodules

Thyroid nodule can develop with solitary or with multiple lesions, and multiple nodules are more common. The multiple nodules can be concentrated in one lobe, but also can be distributed in both lobes and even isthmus. On ultrasonography, multiple

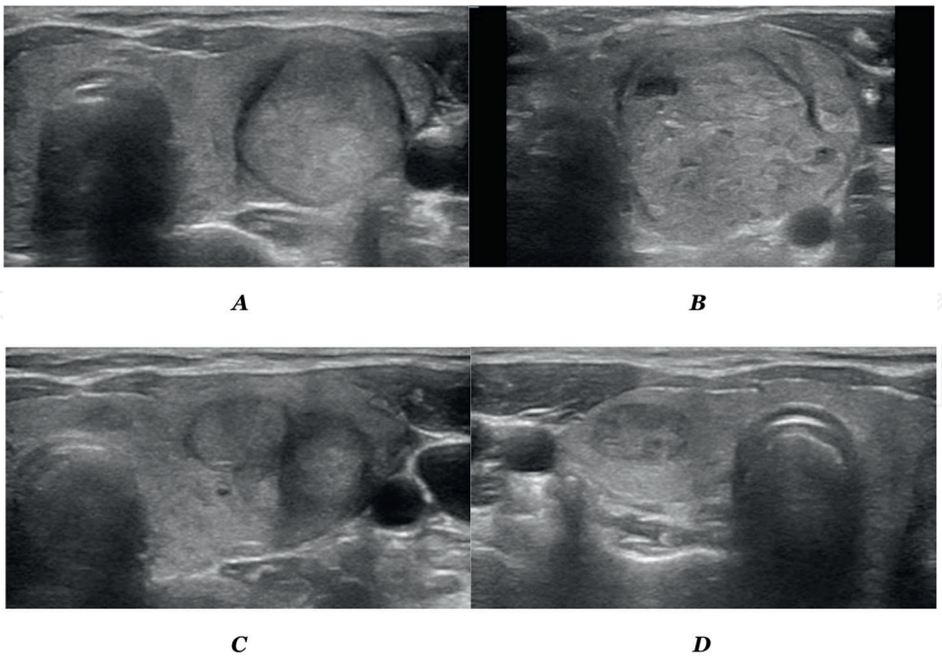


Figure 12.
Multiple thyroid nodules in benign ultrasonic features and all confirmed as benign lesions by FNAC. (A) shows a homogenously isoechoic nodule with intact halo ring at the middle-to-upper portion of right thyroid lobe in a 56-year-old female patient, rating the score of TH-RADS 4a. (B) shows another homogenously isoechoic nodule with incomplete halo ring at the lower pole of right thyroid lobe in the same patient, rating the score of TH-RADS 4a. (C) shows that three nodules were at the middle portion of right thyroid lobe and isthmus in different size and echogenicity in the same patient, rating the score of TH-RADS 4a. (D) shows that one nodule was at the middle portion of left thyroid lobe in homogenous and slightly low echo in the same patient, rating the score of TI-RADS 4a.

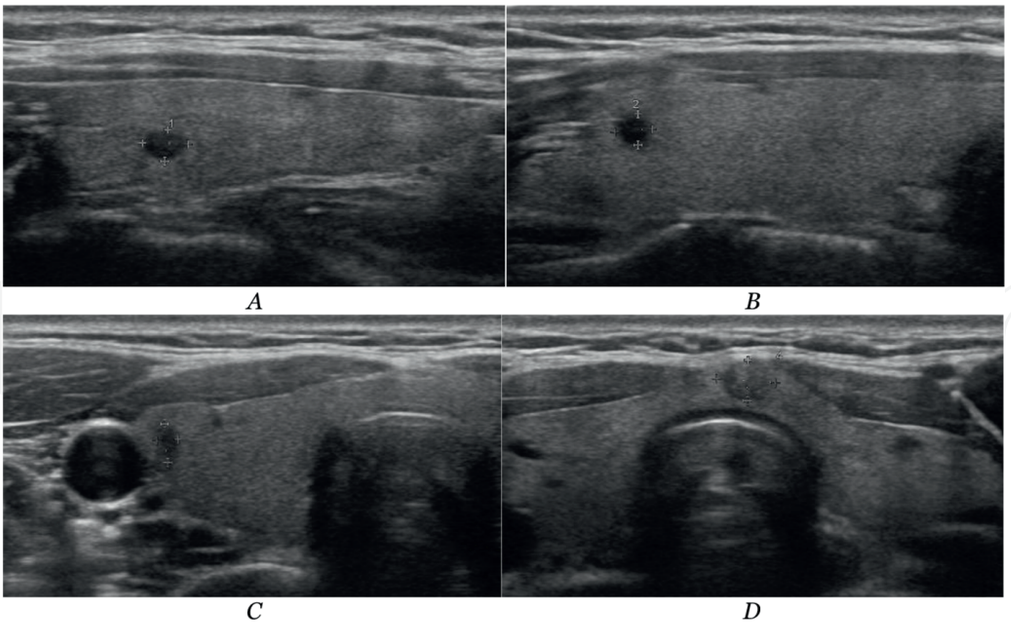


Figure 13.
Multiple thyroid nodules in malignant ultrasonic features and all confirmed as PTC by FNAC. (A) shows that a small hypoechoic nodule was at the upper pole of right thyroid lobe, rating the score of TI-RADS 4b in a 33-year-old female patient. (B) shows that a small hypoechoic nodule was at the upper pole of left thyroid lobe, rating the score of TI-RADS 4b in the same patient. (C) shows that a tiny hypoechoic nodule was at the middle portion of right thyroid lobe, rating the score of TI-RADS 4b in the same patient. (D) shows that a small hypoechoic nodule was at the isthmus of thyroid, rating the score of TI-RADS 4b in the same patient. All the four nodules were eventually confirmed as papillary thyroid microcarcinoma by FNAC.

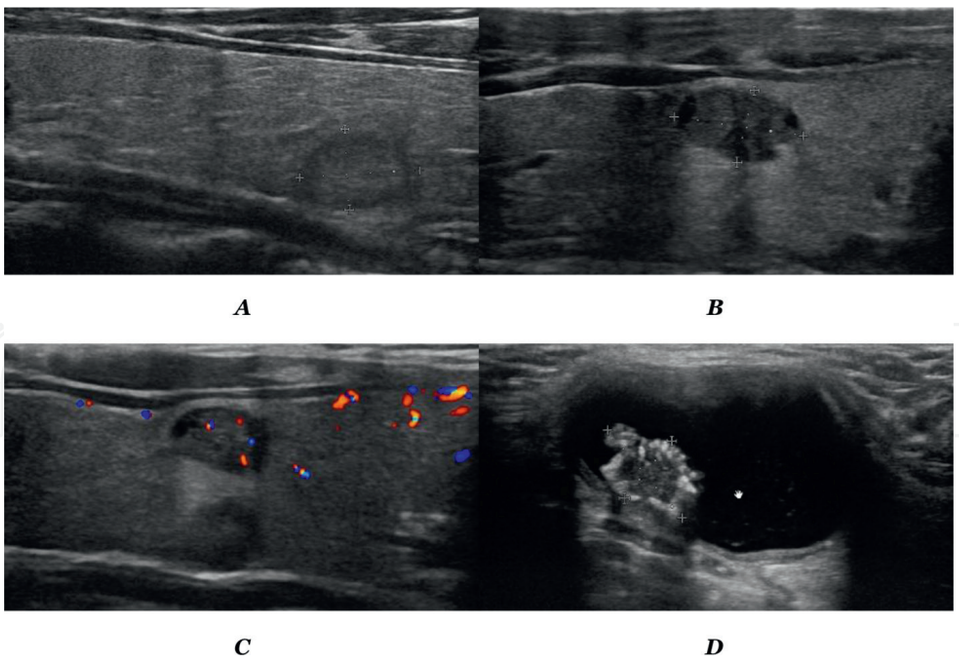


Figure 14.
Multiple thyroid nodules with co-existence of benign and malignant ultrasonic features. (A) shows that a nearly isoechoic nodule was at the middle-to-lower portion of left thyroid lobe in a 41-year-old male patient, rating the score of TI-RADS 4a. FNAC confirmed it as benign thyroid nodule. (B) shows that a hypoechoic nodule was at the middle portion of right thyroid lobe in the same patient, rating the score of TI-RADS 4b. FNAC confirmed it as PTC. (C) shows that two hypoechoic nodules were at the middle portion of left thyroid lobe in the same patient, rating the score of TI-RADS 4a to 4b. FNAC finally confirmed them all as PTC. (D) shows that a cystic-dominant solid nodule was at the isthmus of thyroid with a papillary and calcified solid clump at the bottom of the cyst in the same patient, rating the score of TI-RADS 4b. FNAC finally confirmed the solid nodule as PTC.

nodules may be all in benign ultrasonic features (**Figure 12**) or all in malignant ultrasonic features (**Figure 13**), or part of them may be benign and the rest malignant (**Figure 14**). The critical goal of puncture biopsy is to identify malignant tumors. Therefore, puncture biopsy should be performed on both the nodules in benign ultrasonographic features and the nodules in malignant ultrasonographic features. Only biopsy of nodules with ultrasonographic features that are subjectively considered to be malignant, while ignoring

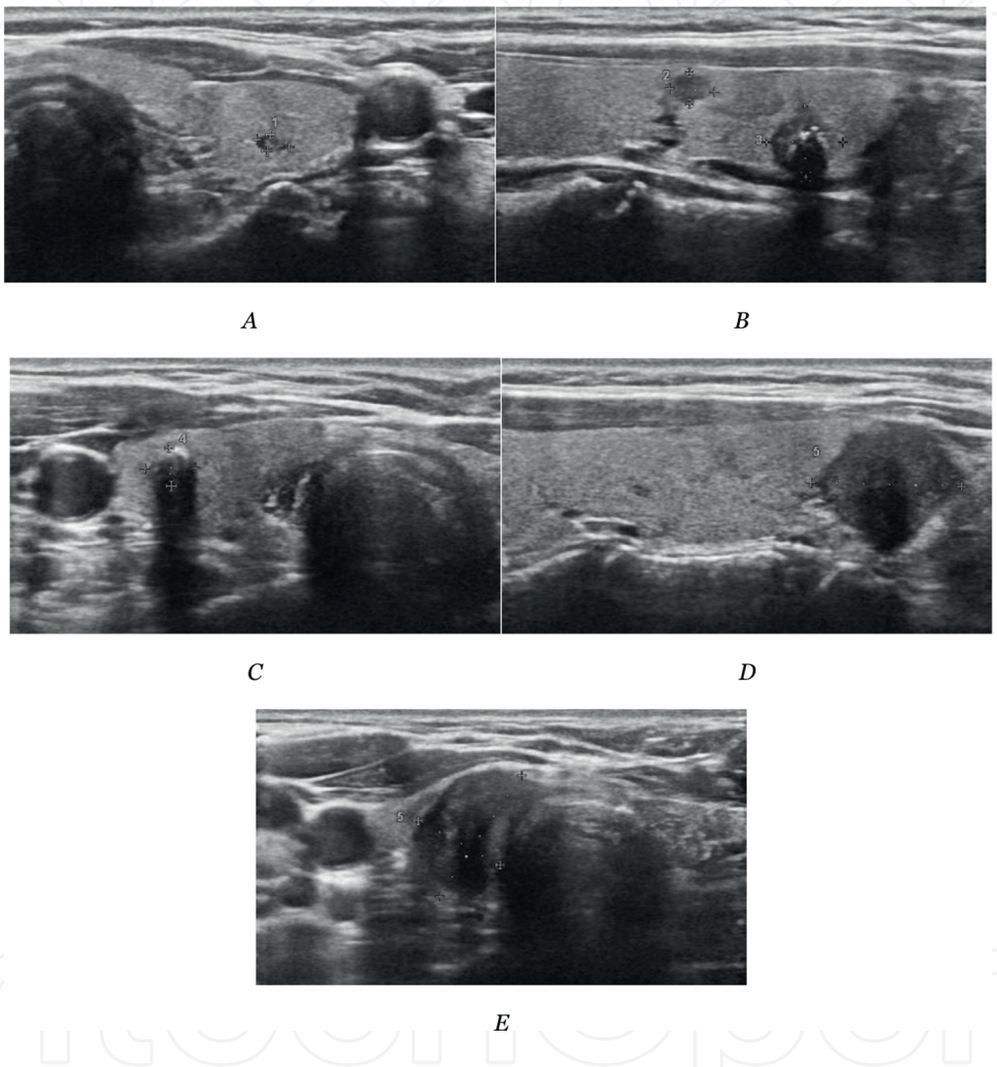


Figure 15.
Multiple malignant nodules with single benign nodule in bilateral thyroid lobes. This patient was firstly admitted at a certain hospital in Shanghai city. Ultrasound-guided FNAB was conducted only on the fifth nodule, while no FNAB was performed on the rest nodules in her thyroids. After FNAC confirmed the fifth nodule as PTC, she came to our hospital to seek for thermal ablation treatment of her PTC. By a detailed ultrasonographic examination, we found that four of her five nodules were highly suspicious of PTC, and the fifth nodule was highly adhesive to the trachea. With informed consent, we conducted FNAB on all her five thyroid nodules and liquid separation test on the fifth nodule in pre-ablation phase. As a result, FANC confirmed that nodule 2 through nodule 5 was of PTC and nodule 1 was of benign lesion, while liquid separation test confirmed that the nodule 5 was tightly adhesive to trachea. Her thyroid conditions were contraindicated to thermal ablation therapy, and she was transferred to Department of thyroid surgery. (A) shows that a solid-dominant cystic nodule (the nodule number 1) was at the upper pole of left thyroid lobe in a 55-year-old female patient, rating the score of TI-RADS 3. (B) shows two solid nodules at the middle portion of right thyroid lobe in the same patient, rating the score of TI-RADS 4a (the nodule number 2) and 4c (the nodule number 3), respectively. (C) shows a entirely calcified nodule (the nodule number 4) at the middle to lower portion of right thyroid lobe in the same patient, rating the score of TI-RADS 4b. (D) shows an extremely hypoechoic nodule at the lower pole of right thyroid lobe in the same patient, rating the score of TI-RADS 5. (E) shows the nodule in Figure D closely adjacent to the trachea in high suspicion of adhesion to trachea on the transverse section.

biopsy of some nodules with ultrasonographic features that appear to be benign, will most likely miss the underlying malignancy (**Figure 15**). This practice is undesirable, because some malignant tumors can present as benign in ultrasonic characteristics. Unfortunately, the practice to biopsy only the nodules with obvious malignant acoustic features for multiple nodules or only one of the multiple nodules with malignant acoustic features is still popular. Such disposal is not conducive to comprehensively and accurately recognizing the patient's condition before treatment and formulating reasonable surgical plans. If the patient is to be treated with non-total thyroidectomy surgery, such as lobectomy, tumor resection, or even thermal ablation, disposal like this is unfavorable for predicting the postoperative progress of the disease too. Comprehensive puncture biopsy for multiple nodules can not only prevent the above problems, but also improve the ability of sonographers to predict the pathologic diagnosis based on the ultrasonic characteristics of nodules and promote the optimization of diagnostic research. In addition, it can also meet patients' need to know the final pathological diagnosis of multiple nodules.

4.2 FNAB ought to be performed on all nodules suspected of malignancy

Whether surgical resection or thermal ablation is recommended for patients with papillary thyroid carcinoma (PTC), the prognosis of PTC is the focus of high attention of both doctors and patients. The number of cancer foci during initial treatment has a significant impact on the adverse prognosis of cervical lymph node metastasis and/or intraglandular recurrence after treatment. When total thyroidectomy is no longer the only treatment option, especially when thermal ablation has become an increasingly popular treatment option, if FNAB is not fully applied to all the multiple suspected PTC foci, it is impossible to accurately define the risk degree of patients' disease before treatment and to accurately judge their prognosis. Therefore, in the face of multiple suspected thyroid papillary carcinoma nodules appearing at the same time, biopsy sampling must be done one by one, and definite pathological diagnosis results must be obtained for each nodule. If the pathological diagnosis is confirmed to be two or more multiple papillary carcinomas, preoperative communication with the patient should be truthful to warn of the risk of poor prognosis. It not only helps patients and their families to warn themselves in advance but also facilitates the high coordination between doctors and patients to avoid the breeding of discontent from patients after operation.

4.3 FNAB ought to be performed on benign nodules with coexisting benign and malignant sonographic features in those who choose thermal ablation therapy

At present, thermal ablation of thyroid nodules is in the initial stage of application and popularization, and the standardization of technical operation has not been generally implemented. In the face of multiple nodules, some medical institutions do not perform puncture biopsy for each thyroid nodule, and ablation treatment only targets malignant nodules that have been confirmed by pathological diagnosis. Nodules with benign sonographic appearance but not confirmed yet by pathology were not treated simultaneously. Under such therapeutic strategies, the treatment protocol would lack foresight and holistic view and may lead to patients' dissatisfaction or even disputes.

4.4 FNAB of malignant nodule prior to the benign one in order

On the premise of paying attention to comprehensive puncture biopsy of benign and malignant nodules, attention should be paid to their puncture sequence. For

puncture biopsy, the key issue that both doctors and patients pay attention to is the final pathological diagnosis results of suspected malignant nodules in ultrasonic images. Therefore, the proper order of puncture sampling should be to complete the sampling of acoustically malignant nodules first, then to perform sampling of acoustically benign nodules, so that such a strategy can not only consider the comprehensiveness of the biopsy but also highlight the key concerns.

4.5 Keeping alert on the factors reducing ultrasonic visibility of tiny nodule

4.5.1 Influence factors originating from the rapid glandular swelling

FNAB can cause several fissure-like sonographic changes in thyroid tissue instantaneously, accompanied by gland swelling and boundary blurring of nodular lesions (**Figure 16**), making the piercers lose clear puncture targets. Glandular swelling usually

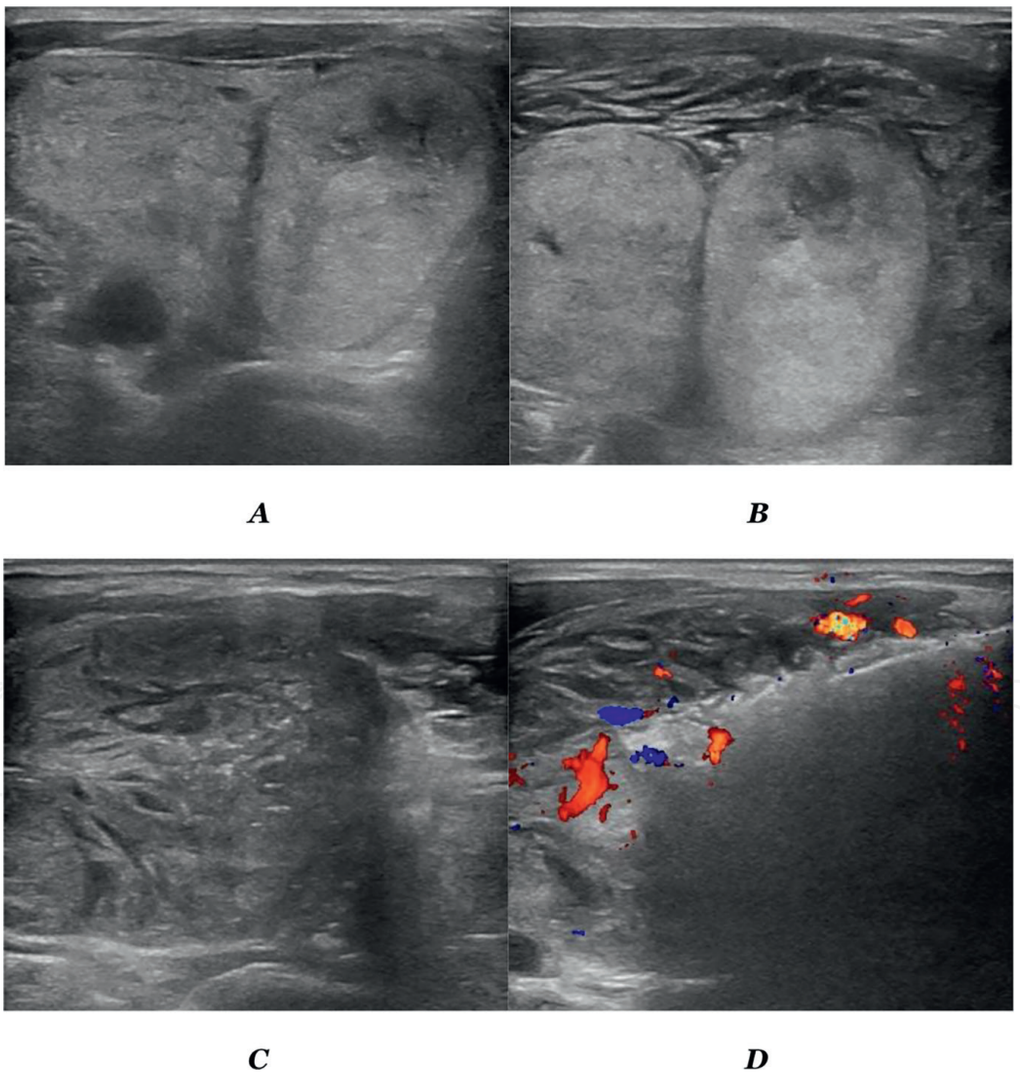


Figure 16.
Feature of glandular edema induced by FNAB. (A) shows that two large nodules scoring TI-RADS 4a were at the left thyroid lobe in a 42-year-old female patient shortly before undergoing FANB and microwave ablation treatment. (B) shows edema developed shortly after implementation of FNAB in the peri-nodule glandular tissue in appearance of numerous fissure-like anechoic areas. (C) shows immediate starting of microwave ablation of the large nodules. (D) shows the display of target nodules got blurred under impact of edema and MWA was ceased due to loss of definite target.

occurs in the gland on the biopsy side, but it can also spread to the isthmus or even to the contralateral glandular lobe. It occurs suddenly and progresses rapidly. In most situations, neither cloudy echoes originated from the red cells deposited due to bleeding on gray-scale ultrasonography nor signals of active bleeding on CDFI scanning can be detected within the fissure-like area. The author speculated that the mechanism of this swelling phenomenon might be that thyroglobulin in follicular cavity oozed out of the follicle along the puncture needle path. This thyroglobulin stimulated sympathetic nerve endings in the glands and caused neurohumoral reflex, and serotonin in thyroid microvasculature oozed rapidly from the vascular cavity into the tissue space to form the fissures. In rare cases, the puncture needle may pierce the tiny blood vessels in the gland and cause bleeding in the interstitial space. Although the incidence of rapid glandular swelling is not high, once it occurs, the ultrasonographic definition of small target nodules is seriously reduced, which greatly affects the accuracy of FNAB procedure. So far, there has been no definite method to prevent this swelling event. Only correct puncture sequence can eliminate its adverse effects on FNAB.

4.5.1.1 Micro-nodules ought be preferentially punctured when multiple puncture targets coexist

Once edema occurs in the glands, the boundary of nodules will be blurred quickly. Among multiple nodules of various sizes, small nodules especially those less than 1 cm will be affected by edema most significantly. Edema can compress small nodules to become smaller. Edema can blur the boundary of small nodules to decrease their visibility. The joint result is that the small target nodules can easily lose their definite positioning, leaving FNAB or MWA off-target. Therefore, to prevent unexpected events, puncture of small nodules should be given priority in specific operations, followed by puncture of small nodules, medium nodules, and large nodules in order. This strategy ensures that all target nodules can be punctured even in the event of glandular edema.

4.5.1.2 Hypo-vascular nodules ought be preferentially punctured when multiple puncture targets coexist

Although bleeding is not believed the primary cause of glandular edema, it can also reduce the ultrasonographic visibility of micro-nodules of thyroid. Therefore, it is necessary to give priority to puncture the hypo-vascular nodules according to the blood supply shown by CDFI of each nodule.

4.5.2 Influence factors originating from the medial margin of sternocleidomastoid muscle

When the thyroid is scanned in transverse section, the medial margin of the sternocleidomastoid muscle is prone to side echo loss effect due to its curved shape, and an echo blind area like acoustic shadow is formed behind it. The blind area is usually not wide but deep and often runs through the transverse section of the thyroid, seriously reducing the clear display of the thyroid nodule located in the blind area, especially the microcarcinoma foci adjacent to the anterior thyroid capsule. It is not uncommon to miss a diagnosis of microcarcinoma. The method to avoid or reduce the lateral echo loss effect originating from the medial margin of the sternocleidomastoid muscle is to adopt unconventional longitudinal scan, that is, the probe acoustic beam is tilted outwards at a large angle, as close to the horizontal plane as possible, and

avoid the sternocleidomastoid muscle. While FNAB is conducted, liquid isolation method can be used to reduce the echo loss effect at the medial edge of sternocleidomastoid muscle and eliminate the blind area.

4.5.3 Influence factors originating from liquid isolation method

Liquid isolation method was originally created to enhance the safety of thermal ablation of thyroid nodules and prevent the adjacent organs and tissue structures around the thyroid from being scalded. However, after continuous optimization and expansion, it has now played an important role in the implementation of FNAB and CNB in thyroid nodules, parathyroid nodules, and cervical lymph node metastases. However, the liquid isolation method has a "double-edged sword" effect. Taking thyroid nodule FNAB as an example, it can improve the clarity of thyroid nodule display and enhance puncture accuracy, which is its positive effect. It can also reduce the visibility of the target nodules or even cause the target to be lost, which is its negative effect. Whether positive or negative effects occur depends on the size, hardness, and location of the lesion, as well as on the amount of liquid spacer injected. As mentioned above, when scanning the thyroid in the transverse section, echo-blind areas were formed in the thyroid section due to the lateral echo loss effect from the medial edge of the sternocleidomastoid muscle, which reduced the clear display of tiny nodules located in the blind area. By injecting isolation agent into the anterior thyroid space to form a certain width of isolation zone, the sternocleidomastoid muscle will be displaced anterolaterally (ventral side), and the thyroid gland will be displaced backwards (dorsal side), so that the thyroid nodule will be removed from the echo loss area. As the isolation agent to be used is saline or hyaluronate gel, echo enhancement effect will yield behind the isolation zone. Under this effect, the definition of the thyroid nodule will be significantly improved. However, it should be noted that if the nodules are relatively small, soft in texture, and the amount of isolation fluid injected is relatively large, the nodules may be compressed and lose their original ultrasonic manifestations, resulting in target blur or even target loss. Thus, how to make good use of the positive effect of liquid isolation method and avoid its negative effect is bound to require FNAB operators to balance various influencing factors.

4.6 Keeping alert on the influence of nodule retreat against puncture

Some small and soft nodules, or some small and hard with ring-calcification nodules, tend to retreat while the needle tip approaches. The retreating phenomenon makes it difficult for the puncture needle to successfully enter the nodule. When these nodules are located at the edge of glands, their retreat will be more significant. Increasing the puncture force and needle speed can improve the hit rate, but the operator will inevitably avoid using force and fast needle insertion due to the fear of puncture needle injury to the common carotid artery, trachea, and esophagus. In this case, if the position of thyroid nodule can be fixed with the help of external forces and the degree of retreat can be reduced, it will be beneficial for puncture needle to penetrate the nodule to obtain specimens. Liquid isolation method is an effective means to form the external forces. By liquid isolation method, the liquid zone gets certain tension, and the tension is higher with the more liquid is injected. The liquid tension can maintain a greater and more lasting external forces to fix the thyroid nodule to reduce its retreat, especially when sodium hyaluronate gel is used as isolation agent. Taking the soft nodules located beneath the lateral thyroid capsule as an

example, the isolation zone in the lateral thyroid space forms a large tension between the common carotid artery and the lateral thyroid capsule, which not only has a "drive away" effect on the common carotid artery to avoid being injured by the puncture needle and improve the puncture safety, but also has a "backrest" effect on the thyroid nodules. This "backrest" effect makes the retreat amplitude of nodules significantly reduced during puncture.

4.7 Avoiding puncture approach directly through the thyroid capsule invaded by suspected PTC foci

Although it is very rare for FNAB of PTC to cause cancer cells to grow along the needle path and then cause tumor to spread out, it is possible for cancer cells to escape from the primary cancer focus and implant in the needle path with the withdrawal of the needle. It is just that most of the time, those cancer cells that shed do not develop into new cancer foci that can be shown by high-frequency ultrasound. To reduce the escape of cancer cells into peri-thyroid space as much as possible, the puncture route should avoid the thyroid capsule where the cancer focus has invaded. In principle, the puncture needle should enter the target nodule through a certain range of normal thyroid tissue.

4.8 Avoiding needle breakthrough the sternocleidomastoid as much as possible

There are generally two puncture routes for FNAB of thyroid nodule. One is R1, which means the needle advances from medial to lateral, and the other is R2, which means the needle advances from lateral to medial [10]. The R1 route is usually preferred, where the puncture needle enters the side lobe of thyroid by the cervical alba or strap muscle, or the isthmus. The R2 route is not preferred because the puncture needle must go through the sternocleidomastoid muscle before entering the thyroid lobe. In the neck area, the sternocleidomastoid muscle is the thickest and most powerful in contraction. The thick muscle has a large binding force on the puncture needle, causing it not convenient to adjust the advancing direction of the puncture needle. The powerful contraction of the muscle can cause the needle to deflect in advancing direction. Moreover, there are abundant blood vessels within the muscle, but some of them are potentially not open, and there is no active blood flow through them, making it difficult to be identified even by scanning with CDFI. These latent blood vessels might have been hit by the puncture needle without being aware of it. Unfortunately, it is not a rare case that hematoma was formed in the sternocleidomastoid muscle after needle extraction. Moreover, the middle thyroid vein emanates laterally from the glandular lobe and is prone to be incidentally injured from puncture needles to form peri-thyroid hematoma while R2 route was taken. For these reasons, it is recommended to take R2 route as less as possible to avoid puncture across the sternocleidomastoid muscle.

4.9 Advocating simultaneous puncture biopsy of lymph nodes suspected of cancer metastasis

Thyroid malignancies are easy to metastasize to cervical lymph nodes, and the risk of PTC metastasis to cervical lymph nodes is much higher than that to distant organs. For PTC, whether surgical resection or ultrasound-guided thermal ablation is adopted, determining whether there is cancer metastasis in cervical lymph nodes before surgery is obliged, because it is not only directly related to whether lymph nodes should be included in the treatment plan, but also related to more accurate

prediction of postoperative progress of patients. Patients should be clearly informed of the illness before surgery so that they can adjust their psychological expectations as much as possible. However, in some medical institutions, it is not uncommon to only take biopsy samples from thyroid lesions with disregarding of lymph nodes. Therefore, it is indeed necessary to enhance the overall working concept and perform puncture biopsy on suspected malignant nodules of thyroid and suspected metastasis of cervical lymph nodes at the same time.

4.9.1 Liquid isolation method should be used too in lymph node biopsy to improve safety or optimize puncture path

Cervical lymph nodes were widely distributed from region I to region VII, and most of them were adjacent to arteries, veins, trachea, esophagus, and nerves. These important structures have potential influence on the safety of puncture operation. When performing FNA on lymph nodes, liquid isolation method can not only optimize the puncture path, making the target lymph nodes move to the ideal puncture path, but also drive the above adjacent structures leave the lymph nodes, improving the safety of the puncture operation.

4.9.2 The puncture needle must avoid the common carotid artery, transverse carotid artery, inferior thyroid artery, and internal jugular vein

Lymph nodes in region II, III, and IV are concentrated in the common carotid artery sheath. Around the beginning of the transverse carotid artery is located the region IV cervical lymph nodes, while near the inferior thyroid artery is located the region VI lymph nodes. All these lymph nodes are high incidence sites of PTC metastasis. To conduct cervical lymph node puncture biopsy, it is necessary to be familiar with the ultrasonic image features of the above vessels, especially the transverse carotid artery, and be skilled in separating lymph nodes from them by fluid isolation method.

4.9.3 Lymph nodes behind the internal jugular vein should not be sampled through venipuncture after venous closure by probe compression

The posterior compartment of the internal jugular vein is a common location of deep cervical lymph nodes, and lymph node metastasis in this location is not rare. Possibly due to the low wall tension of the internal jugular vein, the vascular cavity is easy to collapse after compression, so it has been reported that the internal jugular vein (IJV) is compressed to collapse, and the puncture needle penetrates the vein to reach the deep cervical lymph node to obtain sample materials. Although it may not cause serious bleeding, the rationale for this performance was questionable because it was not certain whether the tumor cells were artificially introduced into the bloodstream. The proper way to conduct biopsy of the lymph nodes deep to the IJV is to make the lymph nodes separated from the vein to form a safe and accessible puncture path under the aid of fluid isolation technique.

4.9.4 The target lymph nodes should be clearly differentiated from the transverse plane of cervical plexus and brachial plexus

The transverse plane of cervical plexus and brachial plexus is almost echoless. Lymph nodes in zone II are more adjacent to cervical plexus, and lymph nodes in zone

V are more adjacent to brachial plexus. Target lymph nodes should be clearly distinguished from cervical plexus and brachial plexus to avoid accidental injury of nerves by puncture needles.

4.10 Clearly labelling whether FNA samples taken before or taken after thermal ablation

Using ultrasound-guided thermal ablation is the new trend in treatment of benign thyroid nodules, Grave's thyroid disease, papillary thyroid carcinoma, and associated cervical lymph node metastasis. Pre-ablation puncture biopsy to obtain pathological diagnosis is the key step to perform thermal ablation therapy. Core needle biopsy (CNB) especially FNAB can be commonly applied for various thyroid diseases. However, some of thyroid nodules are rich in blood supply, some are rich in colloid component, and this structural performance can reduce the quality of biopsy samples or can cause severe bleeding. As a countermeasure, thermal ablation can be performed before the puncture biopsy to block the blood supply to the target lesion and coagulate the target tissue. After this management, the quality of biopsy samples can be raised, and bleeding can be reduced. For example, for the target nodules with plentiful blood supply, heat blocking the nourishing blood flow of the lesion first (only blocking the blood flow without coagulating the nodular tissue) before puncture biopsy is beneficial to reduce the blood composition of the specimen and reduce the bleeding in the puncture needle path. For follicular nodules rich in gelatinous content, it is beneficial to improve the specimen shaping degree and facilitate pathological examination. However, the morphologic changes of the cells occurred immediately after heat and dehydration, resulting in appearance of short rod or spindle cells. When biopsy material is submitted to the pathologist, it is necessary to clearly claim that the biopsy material is taken after block of blood supply or after coagulation of the nodular tissue, otherwise the cell morphology seen under the microscope may easily cause the pathologist to misjudge the nature of the nodules.

4.11 Strongly advocating determination of whether the cancer foci of thyroid gland are adherent with adjacent structures during the process of FNA

Nodular goiters and adenomas are rarely adhesive to surrounding structures, no matter how large they are. However, once thyroid malignancies and inflammatory nodules break through the thyroid capsule, the risk of adhesion to adjacent structures is greatly increased. Adhesion can make the thyroid gland and adjacent structures involved in each other, which can cause the suffer to develop irritation cough, foreign body sensation during swallowing. Severe adhesion can cause failure to separate the thyroid gland from the adjacent structure and failure to remove the lesion in surgical resection as well. Adhesions can cause even more adverse effects on the thermal ablation process of thyroid because surgical instruments such as curets and separators cannot be used in the thermal ablation to separate the adhesions. Only the tension formed by the liquid separator can only be relied on to promote the separation of adhesive structures. Adhesion not only affects the completeness and thoroughness of the lesion ablation, but also may cause serious consequences by scalding the tracheal wall, the esophageal wall, the nerve, and other important structures due to thermal radiation. Therefore, for PTC, whether surgical resection or thermal ablation is taken, it is of great guiding significance for the formulation of appropriate treatment plan to determine whether the cancer foci and adjacent structures are adhered together and the degree of adhesion before operation.

The primary intention of FNAB is to obtain thyroid lesion samples for cytological examination and diagnosis through fine needle puncture, which is a routine means to achieve preoperative pathological diagnosis of papillary thyroid carcinoma. Cervical liquid isolation method is a routine protective measure used in the process of thyroid nodule thermal ablation. The protective effect of the method is achieved by separating the thyroid gland from the surrounding structures with fluid. The fundamental marker for determining adhesion is whether the thyroid gland can be separated from the surrounding structures. Therefore, the liquid isolation method when performing FNAB can not only help to achieve the purpose of cytological examination, but also help to determine whether the thyroid lesion and adjacent structures are adhered to, which can be said to kill two birds with one stone.

4.11.1 For the carcinoma focus to be treated with thermal ablation

Papillary carcinoma nodules are prone to occur beneath the thyroid capsule. A retrospective analysis of a group of 955 patients with PTC treated by microwave ablation showed that the incidence of cancer lesion invading the thyroid capsule was about 41.97% (396/955), and the ratio of invasion of the thyroid capsule ranged from 15.11 to 92.54%.

4.11.1.1 For the carcinoma focus adjacent to trachea

1. For the carcinoma focus located at the isthmus

The isthmic thyroid gland is thin, and the posterior capsule of this glandular tissue is immediately attached to the anterior wall of the trachea all the dimension. Therefore, the cancer nodules located in the isthmus are highly likable to break through the posterior capsule and approach the trachea even if they are small. In a group of 14 patients with PTC that was not suitable for thermal ablation, seven cases were due to adhesion of the cancer focus to the trachea, among which the cancer focus of five patients was in the isthmus. Three cases of the isthmus-located cancer were found to get the lesions 1 year ago, but they were not treated in time. While they came to our center for thermal ablation therapy, the cancer foci were found being closely adhered to the trachea, liquid isolation method could not separate the cancer focus away from trachea. Therefore, the author suggests that attention should be paid to early diagnosis and treatment of papillary thyroid carcinoma located in the isthmus. Once found, puncture biopsy is performed, and once diagnosed, thermal ablation is performed.

2. For the carcinoma focus located beneath the medial capsule of thyroid

The medial thyroid capsule is adjacent to the lateral wall of the trachea, and the cancer focus under the medial capsule breaks through the capsule and is easy to adhere to the trachea. Among the above seven patients whose cancer foci adhered to the trachea and failed to undergo thermal ablation, the cancer foci of the other two patients were located just beneath the medial thyroid capsule, but all of them were non-micro carcinomas with a diameter greater than 1 cm.

4.11.1.2 For the carcinoma focus adjacent to the esophagus

In most cases, the esophagus is located on the left side of the trachea, descending behind the left thyroid gland. The anterior wall of the esophagus is adjacent to the posterior thyroid capsule, and there is little fatty tissue filling between them. Therefore, cancer foci under the posterior capsule of thyroid can potentially invade the esophagus while breaking through the capsule. Through swallowing action, tumor infiltration adhesion can be initially judged on real-time ultrasonography, but it can be more convincingly determined through liquid isolation method. But so far, the author has not found the thyroid cancer foci closely adhered to the esophagus.

4.11.1.3 For the carcinoma focus adjacent to the strap muscle

There is a risk of carcinoma closely beneath thyroid capsule to break through the capsular membrane and infiltrate the strap muscle. The mutual pulling between thyroid and strap muscle can be seen on real-time ultrasonography during swallowing, and it can be more clearly determined whether there is infiltrating adhesion between them through liquid isolation method. Surgeons would both resect thyroid cancer lesion and strap muscle if the cancer focus has invaded the strap muscle. While thermal ablation is adopted for PTC, both the thyroid cancer lesion and infiltrated strap muscle would be ablated together too. So far, the PTC foci invading strap muscle is not contraindicated to thermal ablation therapy in our center.

4.11.1.4 For the carcinoma foci adjacent to the laryngeal nerve

The laryngeal nerves which are closely related to the surgical treatment of papillary thyroid carcinoma include recurrent laryngeal nerve (RLN) and lateral branch of superior laryngeal nerve (SLN). If the cancer focus has invaded the RLN or the lateral branch of the SLN, it will usually cause changes in the patient's voice, which is often hoarse, deep voice, and reduced volume. However, it is not sufficient to determine whether the laryngeal nerve is invaded by cancer foci only based on the changes of voice, which should be combined with the movement status of vocal cords seen by direct laryngoscopy. The lateral branch of the SLN is adjacent to the upper pole of the thyroid gland, and the entry of the RLN is close to the suspensive ligament of the thyroid gland. Special attention should be paid to the PTC located at the above corresponding position to distinguish whether the cancer focus is adhered to the laryngeal nerve.

1. For the tumor foci adjacent to the entrance of RLN into the larynx

Figure 17a shows the location of the cancer focus near the RLN into the larynx. Fluid is injected into the medial thyroid space to move the thyroid cancer focus laterally as much as possible away from the RLN in the risk triangle (**Figure 17b**). If the lesion is not displaced, it is highly suggested that the lesion is located near the suspensory ligament. Even if there is no adhesion to the RLN, there is still a risk of injury to the RLN during thermal ablation of the PTC focus.

2. For the tumor foci adjacent to the lateral branch of SLN

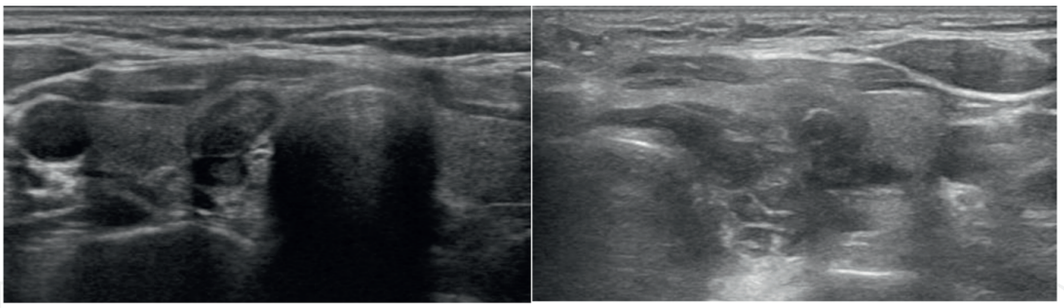


Figure 17.
PTC foci closely adjacent to the entrance of RLN into the larynx. (A) Shows that an irregular hypoechoic nodule was located close to the trachea and entrance of RLN into the larynx; (B) shows that the nodule was driven away from the trachea and entrance of RLN into the larynx.

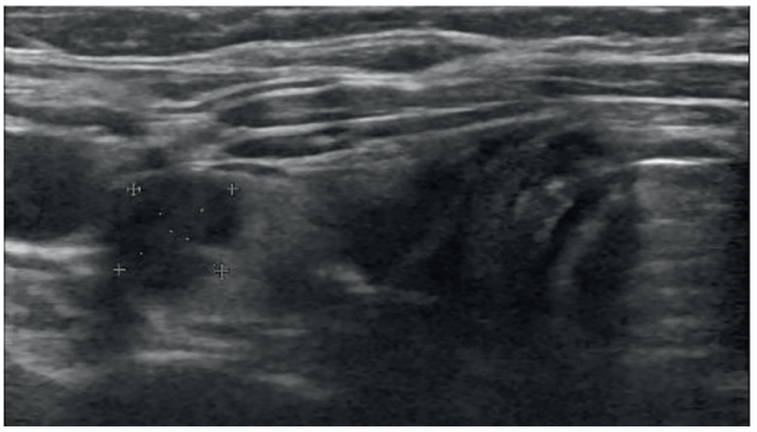


Figure 18.
PTC foci located at upper pole of thyroid.

Figure 18 shows that the cancer focus is on the upper pole of the thyroid gland, which is normally disconnected from the trachea and can be completely separated. If the cancer focus cannot be separated away from trachea after liquid isolation, it can be judged that adhesion developed, indicating there is a risk of injury to SLN during thermal ablation of the PTC focus.

4.11.2 For the ablation areas of thyroid lesions

No matter for PTC or benign thyroid nodules, various degrees of local adhesion may occur after thermal ablation of them. The adhesion of the ablation area to the adjacent structure can also cause irritation, choking, and foreign body sensation during swallowing. Conversely, these symptoms may not necessarily be caused by the ablative adhesions. Therefore, to identify the cause of the patient's discomfort symptoms after ablation therapy, a liquid isolation test could be performed by the chance of post-operative FNAB assessment to determine whether there was local adhesion and the degree of adhesion according to the separability shown on real-time ultrasonography. Adhesion area can present local adhesion to the trachea, the anterior cervical strap muscle, the esophagus, the cervical plexus or brachial plexus and causes relevant signs and discomfort. If the adhesions can be loosened by liquid separation, it will help relieve the discomfort.

4.12 Recommend making identification of hormone secretion status of nodules during FNAB process

The essence of hyperthyroidism is that the gland produces excessive thyroid hormone. The excess hormone can come from diffuse hyperplasia of the gland tissue in Grave's disease or from benign thyroid tumors called functional adenomas. When Grave's disease is associated with adenoma, does the thyroid hormone come from the adenoma tissue or from extra-tumoral gland tissue? Microwave ablation has not only been effective for treating benign thyroid nodular and papillary carcinoma [11–15], but also successfully treated hyperthyroidism [16–19]. Identifying the real source of excess hormones is of great significance for accurate positioning and guidance of thermal ablation in the treatment of hyperthyroidism. Although iodine-131-PET can indicate the presence of rich iodine uptake in the thyroid, iodine uptake is not directly equivalent to thyroid hormone synthesis. The essence of precision ablation therapy is to find the site of excess thyroid hormone synthesis and destroy it, which requires direct sampling of thyroid adenoma and extra-tumoral glands to determine their respective thyroid hormone levels. This can be achieved by thyroid FNA manipulation and preparation of FNA puncture eluent.

Ultrasound-guided puncture biopsy and puncture ablation have become an irresistible new force in the surgical treatment of thyroid nodule, tumor, and other space-occupying diseases. Above, the author's brand-new thinking and analysis on the technical improvement and functional expansion of fine needle puncture of thyroid nodules from nine aspects mainly focus on full preoperative planning and preparation to obtain more perfect treatment results, which is of significance to promote the progress of thyroid surgery and thermal ablation therapy.

5. Conclusion

In this chapter, the authors firstly give an independent description of FNI in the basic technical concept of fine needle puncture biopsy. We aim to remind people not only to pay attention to FNA technology, but also to the function of FNI. This is because FNI is an important support for the implementation of liquid isolation. Liquid isolation method is the core safety protection measure for the thermal ablation of thyroid and other cervical tumors, but its role is much more than that. Over a decade, it has been developed into a special liquid separation method, liquid shifting method, and liquid dissection method and has made a positive contribution to the clinical application and progress of FNAB.

The goal of FNAB is always to obtain sufficient and qualified cellular specimens, while the premise of FNAB is always to keep the target lesion under clear ultrasonic display, and the fine needle can safely enter the target lesion. Based on the structural complexity and diversity of thyroid nodules, the authors establish concepts associated with methods of "9 + X"-needle passages puncture modality, FNAB immediately after heat blocking nutrient arteries, and FNAB immediately after heat coagulation of nodule tissue. These methods have effectively optimized FNAB and FNAC.

In the face of the rapid development of thermal ablation in treatment of thyroid nodules especially papillary carcinoma and related cervical lymph node metastasis, the authors especially suggest that determination of whether the suspected cancer foci are adherent to adjacent structures should be done in meanwhile of FNAB. In addition,

comprehensive FANB for multiple nodules and simultaneous FNAB for cervical lymph nodes with suspected metastasis should be conducted, so that the major illness of the patient's thyroid and cervical lymph nodes can be fully understood through a single FNAB operation to facilitate the patient to make an appropriate and prompt choice between surgical resection and ultrasound-guided thermal ablation for the thyroid diseases.

Last but not least, the author emphasizes that systematic thinking and design are important for premium diagnosis and treatment of thyroid nodules with fine needle puncture.

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Conflict of interest

The authors declare no conflict of interest.

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