

EXPRESSION OF SERUM INFLAMMATORY FACTORS AND VASCULAR ENDOTHELIAL GROWTH FACTOR AND VOLUME REDUCTION RATE IN THYROID NODULES TREATED WITH ULTRASOUND-GUIDED RADIOFREQUENCY ABLATION

EKSPRESIJA SERUMSKIH INFLAMATORNIH FAKTORA I VASKULARNOG ENDOTELNOG FAKTORA RASTA I STOPA SMANJENJA ZAPREMINE ČVOROVA ŠTITASTE ŽLEZDE LEČENIH RADIOFREKVENTNOM ABLACIJOM POD KONTROLOM ULTRAZVUKA

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Summary

Background: The incidence of benign thyroid nodules (TN) is high. Minimally invasive ablation is a commonly used treatment in clinical practice. However, the distinctions in efficacy and safety between different ablation techniques still need to be clarified. This study aimed to compare the effects of ultrasound-guided radiofrequency ablation (RFA) and microwave ablation (MWA) on serum inflammatory factors, vascular endothelial growth factor (VEGF) expression, nodule volume reduction, thyroid function, and coagulation function in patients with benign thyroid nodules.

Methods: One hundred and three patients with benign TN were retrospectively included and allocated to an RFA group (n=58) or an MWA group (n=45). Baseline data and serum levels of inflammatory factors, nodule volume, thyroid function indicators (FT3, FT4, TSH), coagulation function parameters (PT, APTT), and vascular endothelial growth factor (VEGF) expression in nodule tissues pre- and post-surgery were extracted.

Results: No distinctions in baseline data were noted ($P>0.05$). On day 1 after surgery, the levels of inflammatory factors were lower in the RFA group versus the MWA group; The volume reduction rate of the RFA group was higher at each time point after surgery; At 6 months after

Kratak sadržaj

Uvod: Incidencija benignih čvorova štitaste žlezde (TN) je visoka. U kliničkoj praksi se često koriste minimalno invazivne ablativne metode. Međutim, razlike u efikasnosti i bezbednosti između različitih tehnika ablacije još uvek nisu u potpunosti razjašnjene. Cilj ove studije bio je da uporedi efekte radiofrekventne ablacije (RFA) i mikrotalasne ablacije (MWA) pod kontrolom ultrazvuka na serumske inflamatorne faktore, ekspresiju vaskularnog endotelno faktora rasta (VEGF), stopu smanjenja zapremine čvora, funkciju štitaste žlezde i parametre koagulacije kod pacijenata sa benignim čvorovima štitaste žlezde.

Metode: Retrospektivno su uključena 103 pacijenta sa benignim TN i raspoređena u RFA grupu (n=58) i MWA grupu (n=45). Prikupljeni su početni podaci, serumski nivoi inflamatornih faktora, zapremina čvora, parametri funkcije štitaste žlezde (FT3, FT4, TSH), parametri koagulacije (PT, APTT), kao i ekspresija vaskularnog endotelno faktora rasta (VEGF) u tkivu čvora pre i nakon intervencije.

Rezultati: Nisu uočene razlike u početnim merenjima ($P>0,05$). Prvog postoperativnog dana nivoi inflamatornih faktora bili su niži u RFA grupi u poređenju sa MWA grupom. Stopa smanjenja zapremine čvora bila je viša u RFA grupi u svim postoperativnim vremenskim tačkama. Šest meseci nakon intervencije, RFA grupa je imala nižu

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surgery, the RFA group had a lower rate of VEGF-positive cells and a higher proportion of negative + weakly positive cells ($P < 0.05$). The RFA group had a superior response rate (94.83% vs 77.78% in the MWA group) and a lower incidence of adverse reactions (ARs) (13.79%), with faster recovery ($P < 0.05$). On postoperative day 1, thyroid function indicators and coagulation parameters in both groups fluctuated slightly. Still, they remained within the normal range, with no significant difference between groups ($P > 0.05$).

Conclusion: For the treatment of benign thyroid nodules, ultrasound-guided RFA was superior in alleviating inflammatory stress, promoting nodule reduction, and inhibiting VEGF expression. Its safety profile was reliable in protecting thyroid function and maintaining stable coagulation function, demonstrating greater comprehensive efficacy and safety. Thus, it can be considered a preferred minimally invasive treatment option.

Keywords: thyroid nodule, ultrasound, radiofrequency ablation, microwave ablation, vascular endothelial growth factor, volume reduction rate, inflammatory response, coagulation function

Introduction

Thyroid nodules (TNs) are common thyroid lesions in clinical endocrinology and thyroid surgery. The incidence in the general population has been increasing year by year, largely due to the widespread use of high-resolution ultrasound and other imaging techniques that allow more frequent detection of asymptomatic nodules (1, 2). Most patients have no obvious clinical symptoms and are often found incidentally during physical examination or examination for other diseases (1). Based on pathological characteristics, TN can be divided into benign and malignant subtypes. Benign nodules account for more than 90%. Still, some benign nodules may cause compressive symptoms due to enlargement, such as globus sensation and hoarseness, or affect patients' psychological state due to obvious protrusion. Malignant nodules, although accounting for a small proportion, need timely intervention to prevent disease progression and metastasis. Therefore, the standardised treatment of TN has always been a focus of clinical attention (2, 3).

For benign thyroid nodules, the traditional treatment options mostly include surgical excision, active surveillance, and, in some cases, levothyroxine suppression therapy. The latter approach is currently considered a doubtful practice and not strongly advocated. Although surgical resection can directly remove the nodules, it has the problems of large trauma, long postoperative recovery time, and possible complications such as hypothyroidism. For patients with multiple nodules or special nodule locations, the difficulty and risk of surgery are further increased (3, 4). Radioactive iodine

stopu VEGF-pozitivnih ćelija i veći udeo negativnih i slabopozitivnih ćelija ($P < 0,05$). RFA grupa je pokazala višu stopu odgovora na terapiju (94,83% naspram 77,78% u MWA grupi), manju učestalost neželjenih reakcija (13,79%) i brži oporavak ($P < 0,05$). Prvog postoperativnog dana parametri funkcije štitaste žlezde i koagulacije u obe grupe pokazali su blage oscilacije, ali su ostali u granicama referentnih vrednosti, bez statistički značajne razlike između grupa ($P > 0,05$).

Zaključak: U lečenju benignih čvorova štitaste žlezde, radiofrekventna ablacija pod kontrolom ultrazvuka pokazala je prednost u smanjenju inflamatornog odgovora, podsticanju redukcije zapremine čvora i inhibiciji ekspresije VEGF-a. Bezbednosni profil metode je pouzdan u očuvanju funkcije štitaste žlezde i stabilnosti koagulacije, što ukazuje na izraženiju ukupnu efikasnost i bezbednost. Stoga se može smatrati preferiranom minimalno invazivnom terapijskom opcijom.

Ključne reči: čvor štitaste žlezde, ultrazvuk, radiofrekventna ablacija, mikrotalasna ablacija, vaskularni endotelni faktor rasta, stopa smanjenja zapremine, inflamatorni odgovor, funkcija koagulacije

therapy is mainly applicable to patients with nodules accompanied by hyperthyroidism. It has limited efficacy for simple benign nodules. It may also cause irreversible damage to thyroid tissue. Conservative drug treatment can only temporarily control nodule growth. It cannot demonstrably reduce volume or cure them, which cannot meet patients' long-term demand for efficacy (5).

Therefore, exploring a minimally invasive, safe, and efficient treatment for TN has become an important direction. With the rapid integration of medical imaging and minimally invasive techniques, ultrasound-guided radiofrequency ablation (RFA) has been increasingly applied in TN. This technology uses ultrasound imaging for real-time guidance, accurately locates the nodule, and induces coagulative necrosis of the nodule tissue via the thermal effect of radiofrequency energy, eventually leading to nodule shrinkage or disappearance (6). Compared with traditional surgery, ultrasound-guided RFA offers the advantages of minimal trauma, a short operative time, rapid postoperative recovery, and maximal preservation of normal thyroid tissue and function. It has become an important choice in treating benign TN. Vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis. It is critical for the occurrence and development of tumours and benign proliferative lesions. It can promote the formation of new blood vessels in the lesion tissue, providing sufficient nutritional support for nodule growth. Its expression level is closely related to the growth activity and volume changes of the nodule (3, 7).

This study aims to systematically explore the clinical value of ultrasound-guided RFA for TN,

focusing on changes in VEGF expression levels in nodule tissues and the dynamic trend in nodule volume reduction rate. By clarifying the effects of RFA on VEGF expression in TN, the possible therapeutic mechanisms can be revealed. Moreover, long-term follow-up of nodule volume reduction would verify the sustained therapeutic effect of this technology. This provides a theoretical basis and practical reference for the optimisation of minimally invasive treatment schemes for TN and for the formulation of individualised diagnosis and intervention strategies in clinical practice, promoting the development of minimally invasive TN treatment towards greater precision and higher efficiency.

Methodology

Participants

103 individuals who received minimally invasive intervention for TN at Norinco General Hospital from February 2023 to August 2024 were retrospectively included. All patients were diagnosed with benign TN (Bethesda grade II) by preoperative ultrasound combined with fine-needle aspiration cytology (FNAC) and had clear indications for treatment due to large nodules (maximum diameter 1.7–4.5 cm) causing neck compression, globus sensation, or cosmetic concerns. According to the treatment method they received, they were allocated to an ultrasound-guided RFA group (n=58) or an ultrasound-guided microwave ablation (MWA) group (n=45). The participants either declined the surgical procedure or opted for a less invasive alternative, or they were considered to be at an increased risk for perioperative complications due to comorbidities like hypertension and diabetes. Therefore, ultrasound-guided ablation was considered an alternative to surgery. Although these comorbidities were not considered absolute contraindications for thyroid surgery, ablation was considered in this case to avoid trauma and anaesthesia-related complications. All participants had complete clinical information, images, and follow-up results before and after treatment, with a follow-up period of at least 12 months.

Inclusion criteria: 1) Those diagnosed with TN by ultrasound combined with FNAC with a pathological Bethesda grade II–III; 2) Nodule maximum diameter of 1.5–4.0 cm with obvious clinical symptoms (such as globus sensation, neck compression) or cosmetic demands; 3) Age between 20 and 65 years old without severe underlying diseases; 4) Those who refused surgical operation and were willing to receive ultrasound-guided RFA.

In nodules categorised as Bethesda III (atypia of undetermined significance/follicular lesion of undetermined significance), a risk stratification

protocol was applied. All nodules categorised as Bethesda III underwent repeat ultrasonography and a multi-disciplinary approach involving endocrinology, radiology, and surgery. Nodules identified as having features suspicious for malignancy on ultrasound (e.g., markedly hypoechoic texture, irregular margins, presence of microcalcifications, suspicious lymphadenopathy) or those clinically suspicious for malignancy were excluded from ablation therapy and underwent additional diagnostic evaluation or surgical treatment. Only those nodules categorised as Bethesda III and found to be at low risk, compatible with benign disease, underwent ablation therapy. There was no evidence of malignant transformation in these nodules categorised as Bethesda III over a follow-up period of at least 12 months.

Exclusion criteria: 1) Those with malignant nodules (Bethesda grade V–VI) or suspected malignancy without further diagnosis; 2) Those with coarse calcification in the nodule, located behind the sternum or adjacent to large blood vessels/trachea that cannot be safely punctured; 3) Those with coagulation dysfunction, uncontrolled hyperthyroidism, or severe cardiovascular, pulmonary, hepatic, or renal diseases; 4) Pregnant or breastfeeding women, or those with a history of neck radiotherapy or thyroid surgery.

Preoperative assessment and data collection

Extraction of baseline data

The data of the participants were retrospectively extracted from the hospital electronic medical record system (EMR), including gender, age, disease duration (time from nodule detection to intervention), nodule location (left lobe/right lobe/isthmus), nodule component (solid/cystic/mixed, with the proportion of cystic components recorded for mixed nodules), preoperative maximum nodule diameter and volume, and preoperative Adler blood flow grade, to ensure the comparability.

Imaging examination data

The thyroid ultrasound images obtained within 1 week of surgery were retrieved from the hospital picture archiving and communication system (PACS). Participants underwent preoperative nodule assessment using a Philips EPIQ 7C colour Doppler ultrasound device (probe frequency 7–12 MHz), operated independently by two physicians with over 5 years of experience in thyroid ultrasound diagnosis (consensus was reached through departmental case discussion in the event of disagreement). The recorded indicators included: nodule location (left lobe/right lobe/isthmus), size (length A, width B, thickness C, accurate to 0.1 cm), component (solid/

cystic/mixed, with the proportion of cystic components recorded for mixed nodules), margin (clear/ill-defined), internal echo (hyperechoic/isoechoic/hypoechoic), blood flow signal (graded by Adler grade: 0 for no blood flow, I for minimal punctate blood flow, II for moderate linear blood flow, III for abundant reticular blood flow). The two-dimensional and colour Doppler images of the nodule were also stored for subsequent volume calculation and comparison after treatment.

Extraction of laboratory data

Laboratory test results were obtained within 1 week before surgery, including routine blood tests (white blood cell count, platelet count), coagulation function (PT, APTT), thyroid function (FT₃, FT₄, TSH), and serum inflammatory factors (CRP, IL-6, TNF- α). All tests were completed by the hospital laboratory using the Roche Cobas 8000 automatic biochemical analyser, with reference ranges based on the laboratory standards.

Tissue sample retention

The nodule tissue samples retained from preoperative FNAC (all paraffin-embedded tissues or -80 °C cryopreserved tissues archived in the hospital pathology department) and the residual nodule tissue samples obtained by ultrasound-guided puncture 6 months after surgery were retrospectively sorted. VEGF expression was detected using the hospital pathology department's routine immunohistochemical (IHC) method, and IHC-stained slides and statistical reports on positive cell rate were retrospectively extracted.

Treatment plans and operating procedures

The treatments were conventional clinical techniques regularly performed at the Thyroid Minimally Invasive Diagnosis and Treatment Centre of Norinco General Hospital. The operating procedures were retrospectively sorted out as follows:

RFA: The ECO-100C RFA system (20173030678, ECO, Nanjing) was used, equipped with a 17G multi-pole cold-cycle ablation needle (exposed needle tip length 5–12 mm); the guiding ultrasound was a Philips EPIQ 7C colour Doppler ultrasound device (probe frequency 7–12 MHz). **MWA:** The YDM-300 MWA device (20183030291, Yuande, Beijing) was used, equipped with an 18G water-cooled MWA needle (exposed needle tip length 3–10 mm); the guiding ultrasound device was the same as that used in RFA.

Preoperative preparation: The patient was placed in a supine position with a soft pillow under

the shoulders to maintain neck hyperextension. The area was routinely disinfected with 0.5% povidone iodine and covered with a sterile drape. Local infiltration anaesthesia was administered around the puncture site and thyroid capsule with 2% lidocaine injection. A fluid barrier was established by injecting 8–15 mL of sterile normal saline into the space between the thyroid and trachea, and the common carotid artery.

Ablation operation: Under real-time ultrasound guidance, the ablation needle was punctured to a depth of 1/3 of the nodule. The ablation system was activated with an initial power of 35–45 W. The power was adjusted according to the range of the gasification strong echo area displayed by ultrasound (maximum ≤ 60 W). The ablation was performed using a deep-to-shallow, edge-to-centre moving method until the strong echo area completely covered the nodule and extended beyond the edge by 1–2 mm. The ablation time was 8–15 min per nodule. For the MWA, under real-time ultrasound guidance, the ablation needle was punctured to the centre of the nodule. The ablation device was activated with an initial power of 40–50 W. The power was adjusted based on the nodule size (maximum ≤ 80 W). The selected power ranges and upper limits (≤ 60 W for RFA and ≤ 80 W for MWA) were based on the manufacturers' recommendations and our institutional protocol, aiming to achieve effective coagulative necrosis while minimising thermal injury to surrounding critical structures in the neck. A targeted ablation + short-distance moving mode was used, with each ablation position lasting 20–30 s until the strong echo area completely enveloped the nodule. The ablation time was 5–10 min per nodule.

Postoperative management: After ablation, the needle tip was left in place for 15–20 s, then slowly withdrawn. The puncture site was pressed with a sterile gauze for 15–20 min, followed by a cold compress with an ice pack on the anterior neck for 30 min. The patient was discharged after 2 h of observation without discomfort. According to our institutional practice, patients were routinely prescribed oral antibiotics for three days to prevent infection (Amoxicillin capsules, 0.5 g per dose, three times per day). We acknowledge that routine antibiotic prophylaxis is not universally recommended for clean, minimally invasive thyroid procedures; however, this regimen has been adopted as a local protocol at our centre further to reduce the already low risk of post-procedural infection.

Observation indicators and criteria for judgment

All observation indicators were routine follow-up items in clinical diagnosis and treatment. Data were retrospectively extracted from patients'

postoperative follow-up medical records, ultrasound reports, and laboratory test reports without any additional follow-up content:

(1) Serum CRP, IL-6, and TNF- α test results from one day before surgery and one day after surgery were extracted for both groups of patients. Simultaneously, thyroid function indicators (FT3, FT4, TSH) and coagulation function parameters (PT, APTT) were extracted from one day before and one day after surgery to assess the impact of treatment on thyroid and coagulation function.

(2) The thyroid ultrasound reports of the participants before surgery and at 1, 3, 6, and 12 months after surgery were extracted. The length, width, and thickness of the nodule at each time point were recorded. The volume reduction rate was recalculated using the equation: volume reduction rate = $[1 - (\text{follow-up volume}/\text{preoperative volume})] \times 100\%$. If the ultrasound report indicated that the nodule had completely disappeared, the volume was recorded as 0 and the volume reduction rate as 100%.

(3) The IHC staining results of the nodule tissue of the participants before and 6 months after surgery were retrospectively analysed. According to the VEGF-positive cell rate in the original report (number of positive cells/total number of cells $\times 100\%$), it was divided into four levels: negative ($<10\%$), weakly positive (10%–30%), moderately positive (31%–60%), and strongly positive ($>60\%$). The specific value of the positive cell rate was extracted for statistical analysis.

In addition, continuous percentages of VEGF-positive cells were reported in pathology reports. However, for the initial analysis, a semi-quantitative grading system was used: negative, weakly positive, moderately positive, and strongly positive. This system has proven good reproducibility among observers in our pathology department. Moreover, the distribution of percentage values was skewed and not normally distributed. In such a case, it would be difficult to use non-parametric tests.

(4) Clinical efficacy and safety: Efficacy judgment: With 12 months after surgery as the assessment time point, it was divided according to the volume reduction rate: cure ($\geq 90\%$ or nodule disappearance), effective (50%–89%), and ineffective ($<50\%$ or volume increase). The definition of cure as a volume reduction rate (VRR) $\geq 90\%$ was adopted based on previous large clinical studies and international consensus statements on thermal ablation of benign thyroid nodules, in which a VRR $\geq 90\%$ is considered indicative of near-complete ablation and durable clinical success.

The total effective rate = (number of cured cases + number of effective cases)/total number of cases $\times 100\%$. The results of efficacy judgment were extracted from the follow-up medical records

at 12 months after surgery and, if necessary, combined with ultrasound image review. Safety assessment: The records of adverse reactions (ARs) during treatment and the postoperative follow-up period for participants were extracted, including pain (VAS score ≥ 3). Post-procedural pain was evaluated using a 0–10 visual analogue scale (VAS) at follow-up visits or by telephone interview. Pain duration was defined as the number of days from the procedure until the VAS score decreased to <3 without the need for regular analgesic medication, as documented in the medical and nursing records), local hematoma (diameter >1 cm), skin redness and swelling (range >2 cm around the puncture site), hoarseness (lasting >1 week), and thyroid dysfunction (abnormal TSH at one month/3 months post-intervention). The incidence rate of ARs and recovery time were statistically analysed (extracted from nursing records and medical course records).

Statistical processing

SPSS 22.0 software was employed. Normally distributed measurement data were reported as mean \pm SD ($\bar{x} \pm s$) and compared by one-way ANOVA, while those not following a normal distribution were analysed adopting the Mann-Whitney test. Categorical data were reported as frequencies and percentages (%), and the chi-square test was used for intergroup comparisons. A statistical significance threshold of $P < 0.05$ was applied.

Results

Baseline characteristics

There were no statistically significant differences in gender, age, disease duration, nodule location, nodule component, preoperative maximum diameter, preoperative volume, and preoperative Adler grade ($P > 0.05$), with comparability (Table 1).

Serum inflammatory factors

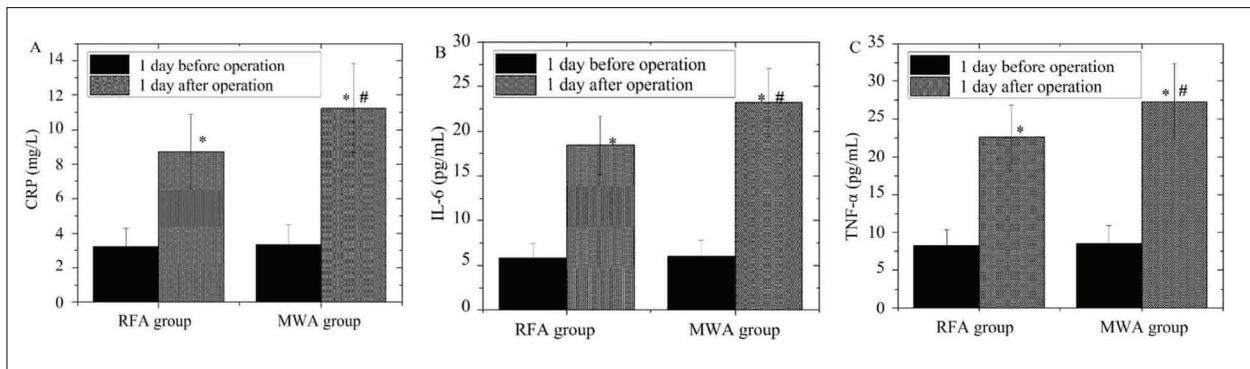
One day before surgery, the levels of serum CRP, IL-6, and TNF- α of the participants were compared ($P > 0.05$). On postoperative day 1, the participants' factors showed an elevation relative to preoperative levels. Still, the magnitude of increase from preoperative to postoperative values was smaller in the RFA group than in the MWA group, indicating a milder systemic inflammatory response after RFA ($P < 0.05$) (Figure 1).

Nodule volume reduction rates at different time points after treatment

At 1, 3, 6, and 12 months after surgery, the nodule volume reduction rates of the participants

Table 1 Contrast of baseline characteristics of participants.

Index	RFA (n=58)	MWA (n=45)	Statistic	P
Gender (cases, %)				
Male	21 (36.21)	17 (37.78)	$\chi^2=0.183$	0.669
Female	37 (63.79)	28 (62.22)		
Age (years)	42.35±8.62	43.17±7.95	t=-0.462	0.645
Duration of illness (months)	11.24±3.58	10.86±3.21	t=0.531	0.596
Location of the nodule (cases, %)				
Left lobe	23 (39.66)	19 (42.22)	$\chi^2=0.572$	0.751
Right lobe	28 (48.28)	21 (46.67)		
Isthmus	7 (12.07)	5 (11.11)		
Component of the nodule (cases, %)				
Solid	32 (55.17)	26 (57.78)	$\chi^2=0.845$	0.655
Cystic	8 (13.79)	5 (11.11)		
Mixed	18 (31.03)	14 (31.11)		
Proportion of cystic components recorded for mixed nodules (%)	35.21±8.74	33.86±7.92	t=0.724	0.471
Preoperative maximum diameter (cm)	2.86±0.65	2.93±0.58	t=-0.548	0.585
Preoperative volume (cm ³)	8.72±3.15	9.05±2.87	t=-0.536	0.593
Preoperative Adlergrade (cases, %)				
0	9 (15.52)	7 (15.56)	$\chi^2=0.913$	0.822
I	25 (43.10)	19 (42.22)		
II	18 (31.03)	15 (33.33)		
III	6 (10.34)	4 (8.89)		

**Figure 1** Contrast of serum inflammatory factors. (A–C represent CRP, IL-6, TNF- α , respectively).

Note: *relative to that one day before surgery, #relative to the RFA group, $P < 0.05$

gradually increased with time; At each time point, the nodule volume reduction rate was noticeably superior in the RFA group versus the MWA group ($P < 0.05$) (Figure 2).

VEGF expression levels in nodule tissue

Before surgery, the contrast between the VEGF-positive cell rate and the distribution of VEGF expression grade in the participants' nodule tissue showed no statistically significant differences ($P > 0.05$). At 6 months after surgery, the

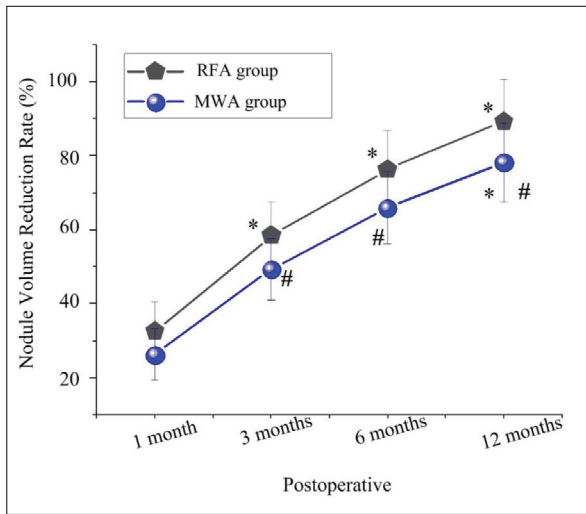


Figure 2 Contrast of nodule volume reduction rates at different time points.

Note: * relative to that one month after surgery, # relative to the RFA group, $P < 0.05$

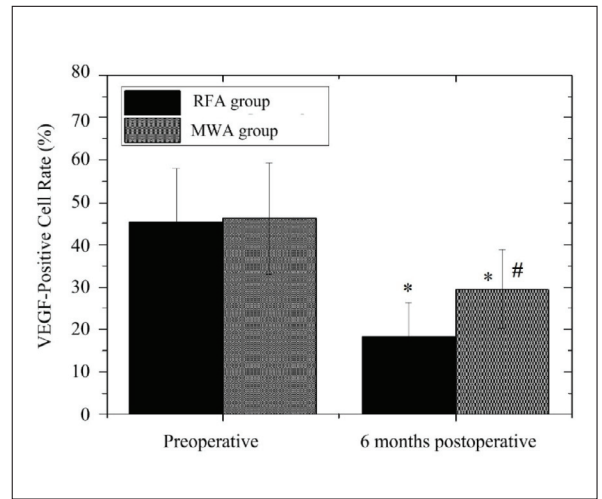


Figure 3 Contrast of VEGF expression levels in nodule tissue.

Note: *relative to that before surgery, # relative to the RFA group, $P < 0.05$

Table II Contrast of VEGF expression grade distribution in nodule tissue.

Time	Group	Negative (<10%)	Weakly positive (10%–30%)	Moderately positive (31%–60%)	Strongly positive (>60%)	Negative + weakly positive (%)	χ^2	P
Before surgery	RFA (n=58)	5 (8.62)	12 (20.69)	28 (48.28)	13 (22.41)	29.31	0.387	0.942
	MWA (n=45)	4 (8.89)	9 (20.00)	22 (48.89)	10 (22.22)	28.89		
6 months after surgery	RFA (n=58)	21 (36.21)	25 (43.10)	10 (17.24)	2 (3.45)	79.31	8.752	0.003
	MWA (n=45)	12 (26.67)	15 (33.33)	14 (31.11)	4 (8.89)	60		

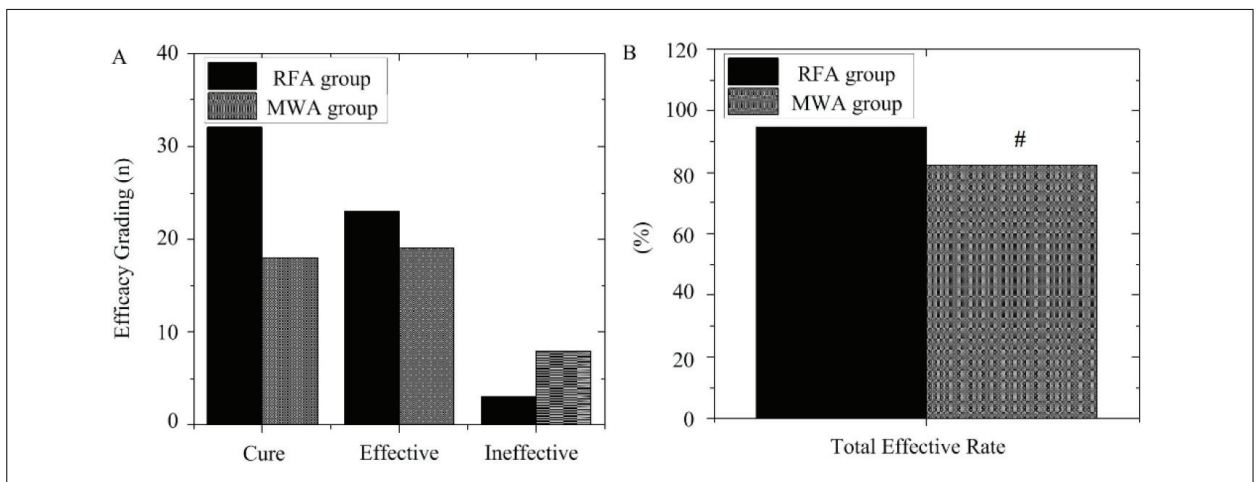


Figure 4 Contrast of clinical efficacy of participants.

(A: Number of cases for cure, effectiveness, and ineffectiveness; B: Total efficacy rate)

Note: # relative to the RFA group, $P < 0.05$

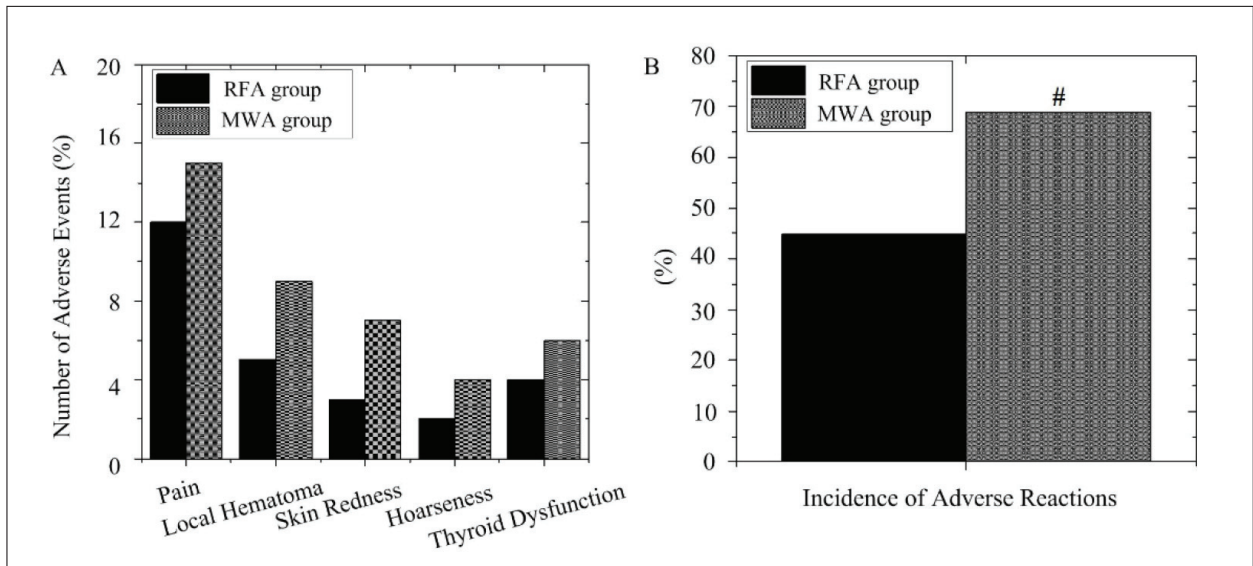


Figure 5 Contrast of ARs occurrence in participants.

(A: Number of cases for pain, local hematoma, skin redness and swelling, hoarseness, and thyroid dysfunction; B: Incidence rate of ARs)

Note: # relative to the RFA group, $P < 0.05$

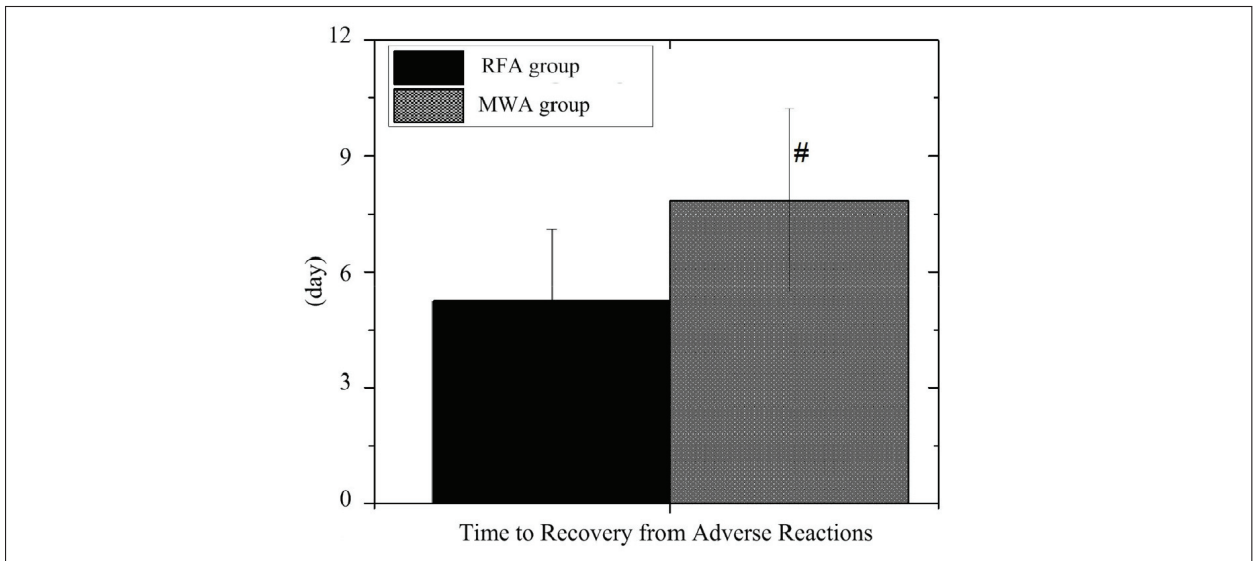


Figure 6 Contrast of recovery time of participants.

Note: # relative to the RFA group, $P < 0.05$

VEGF-positive cell rate of the participants noticeably decreased relative to that before surgery, and the VEGF-positive cell rate was noticeably lower in the RFA group versus the MWA group; In terms of VEGF expression grade distribution, at 6 months after surgery, the proportion of negative + weakly positive was noticeably superior in the RFA group versus the MWA group ($P < 0.05$) (Figure 3, Table II).

Clinical efficacy and safety of participants

At 12 months after surgery, the evaluation of therapeutic effect suggested that the response rate was noticeably higher in the RFA group versus the MWA group; In terms of safety, the incidence rate of ARs was noticeably lower in the RFA group versus the MWA group, with the shorter recovery time of ARs noted in the RFA group ($P < 0.05$) (Figures 4–6).

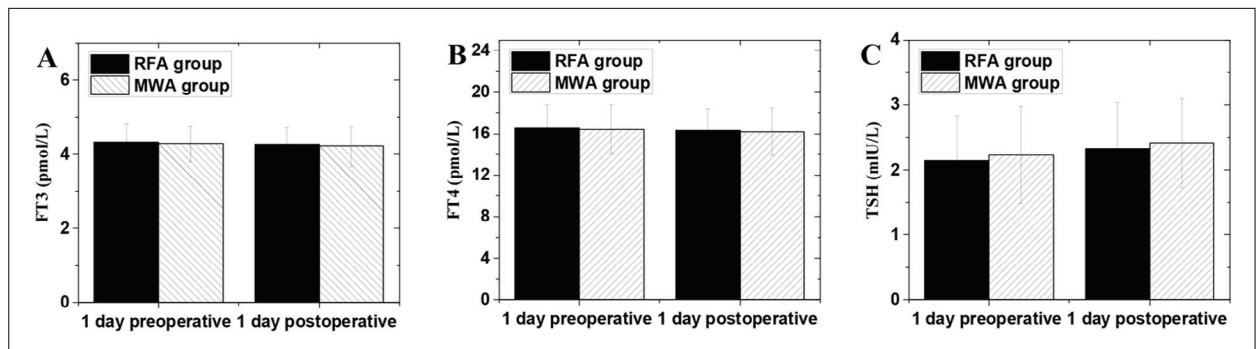


Figure 7 Comparison of thyroid function indicators between the two groups before and after treatment (A–C are FT3, FT4, and TSH, respectively).

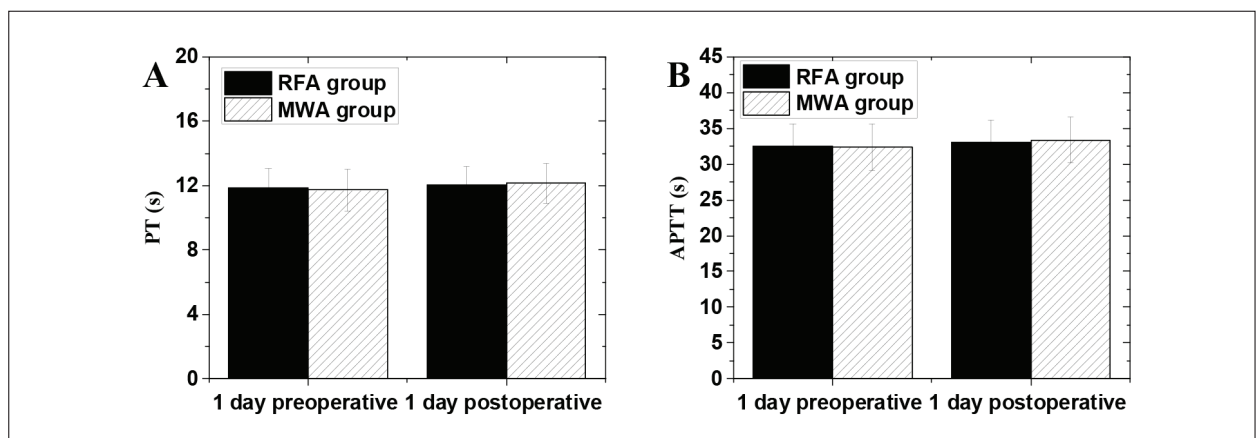


Figure 8 Comparison of coagulation function parameters between the two groups before and after treatment (A–B are PT and APTT, respectively).

Comparison of thyroid function indicators between the two groups before and after treatment

One day before surgery, no statistically significant differences were found in FT3, FT4, and TSH levels between the two groups ($P > 0.05$), indicating a consistent baseline. On postoperative day 1, although the indicators in both groups showed slight fluctuations compared to preoperative values, they were strictly controlled within the clinical normal reference range. No significant difference was observed between groups ($P > 0.05$) (Figure 7).

Comparison of coagulation function parameters between the two groups before and after treatment

One day before surgery, no statistically significant difference in PT or APTT levels was found between the two groups ($P > 0.05$). On postoperative day 1, the parameters in both groups showed only minor fluctuations from preoperative values and remained within the normal reference interval. No

significant difference was found between groups ($P > 0.05$) (Figure 8).

Discussion

This study, by comparing the therapeutic effects of ultrasound-guided RFA and MWA on benign TN, has clarified the technical advantages of RFA, including improved inflammatory responses, enhanced nodule shrinkage, and inhibition of angiogenesis-related factor expression (VEGF). It provides practical evidence for the clinical optimisation of minimally invasive treatment protocols for benign TN. Regarding postoperative inflammatory stress responses, serum CRP, IL-6, and TNF- α levels increased 1 day after surgery. This is directly related to the local tissue damage caused by thermal ablation and the body's stress response. The inflammatory response has been primarily attributed to direct thermal damage to surrounding tissues during ablation, leading to cell membrane damage, protein denaturation, and activation of damage-associated molecular patterns. Furthermore, a transient isch-

emia-reperfusion injury from microvascular occlusion followed by reperfusion may enhance the cytokine cascade, leading to the release of pro-inflammatory cytokines such as IL-6 and TNF- α (8).

However, the levels of inflammatory factors in the RFA group were noticeably lower. The core reason lies in the inherent characteristics of RFA technology: it employs precise temperature control with an initial power of 35–45 W (maximum \leq 60 W). It is combined with a multi-pole cold-cycle ablation needle. This can effectively reduce the diffusion of thermal energy to surrounding normal tissues and minimise non-target thermal damage (2, 9). Meanwhile, basic nursing measures during the operation (such as applying an ice pack to the anterior neck for 30 min) further help alleviate local redness, swelling, and pain responses, thereby indirectly reducing the release of inflammatory factors. In contrast, MWA, with its high thermal efficiency and rapid temperature rise (reaching 100–120 °C), although it shortens ablation time, has a relatively wider thermal diffusion range. Even with the same basic nursing care, it remains difficult to fully counteract the inflammatory response to thermal injury, leading to elevated levels of inflammatory factors (10).

Regarding long-term effects on nodule volume reduction, the RFA group remained superior to the MWA group from 1 to 12 months after surgery. Moreover, at 12 months after surgery, the response rate in the RFA group reached 94.83%, which was noticeably better than the 77.78% in the MWA group. This is closely related to the necrotic effects of RFA on nodule tissue (11, 12).

The RFA response rate of 94.83% is consistent with large series and meta-analytic data on RFA for benign thyroid nodules. The volume reduction ratio-based response rate at 12 months typically approaches or exceeds 90%. Conversely, the 12-month response rate for MWA, at 77.78%, is at the lower end of the reported outcomes range. The outcomes for MWA have been shown to vary depending on nodule composition, operator expertise, and technical protocol. To date, few studies have compared RFA with MWA. However, several reports suggest RFA can achieve a higher rate of volume reduction at longer-term follow-up, particularly in solid-composition nodules (13, 14).

Few studies have specifically compared systemic inflammatory markers after RFA versus MWA for benign thyroid nodules; most comparative reports have focused on efficacy and local complications rather than cytokine dynamics. Our observation that CRP, IL-6, and TNF- α increased to a lesser extent after RFA therefore provides novel evidence suggesting that different thermal profiles of the two techniques may translate into different levels of procedure-related inflammatory stress (15, 16).

Nevertheless, the clinical significance of this difference in response rates (94.83% vs 77.78%) must be viewed with caution. The operator's experience, learning curve effects, and centre-specific proficiency in RFA and MWA may have contributed to the apparent superiority of RFA in this study. It must be validated in further prospective multicentre studies.

RFA achieves coagulative necrosis throughout the entire nodule by uniformly heating the nodule tissue to 60–100 °C through frictional heat generation. The subsequent absorption process of the necrotic tissue is more stable. Meanwhile, standardised postoperative follow-up care (such as regular ultrasound monitoring and dietary guidance) ensures patient compliance. It allows medical staff to monitor nodular absorption promptly. It provides a basis for subsequent interventions (such as supplementary ablation if necessary), thereby further consolidating the therapeutic effect.

In comparison, although MWA has a high thermal efficiency, it is more affected by nodular components (such as cystic components and microcalcifications), which may lead to incomplete local necrosis. In addition, some patients, due to poor follow-up compliance (e.g., failure to undergo regular re-examinations), miss the opportunity for early supplementary treatment, ultimately affecting the long-term volume-reduction effect (17–19). Regarding the safety assessment of thyroid function and coagulation function, this study supplemented the analysis of changes in FT3, FT4, TSH, PT, and APTT before and after treatment in both groups. The results indicated that neither ablation modality caused significant adverse effects on patients' thyroid function or coagulation function. The baseline of relevant indicators was consistent between the two groups preoperatively. Although slight fluctuations occurred on postoperative day 1, all indicators remained within the clinical normal reference range, with no significant intergroup difference. This finding is closely related to the minimally invasive characteristics of both ablation techniques. Ultrasound-guided precise puncture and controlled ablation scope can minimise damage to normal thyroid tissue, avoid thyroid hormone secretion disorders caused by gland destruction, and reduce interference with coagulation-related pathways.

Additionally, intraoperative establishment of a fluid-isolation zone by injecting normal saline effectively protects surrounding blood vessels and tissues, thereby reducing the risk of bleeding. Postoperative standardised pressure haemostasis and cold compress care further ensure the stability of coagulation function. This finding complements the dimensions of safety assessment for both ablation modalities in previous studies, confirming that both RFA and MWA reliably protect thyroid endocrine function and maintain coagulation stability. When combined with the

differences in inflammatory response, nodule reduction efficacy, and adverse reactions mentioned earlier, the comprehensive advantages of RFA in balancing safety and efficacy are further highlighted, providing stronger evidence-based support for the clinical selection of minimally invasive treatment plans.

From a molecular perspective, at 6 months after surgery, the VEGF-positive cell rate in nodule tissue was significantly lower. The proportion of negative and weak-positive cells was higher in the RFA group, indicating that RFA can more effectively inhibit nodule angiogenesis. As a key regulator of angiogenesis, VEGF expression levels directly affect the nutritional supply to the nodule.

A more marked decline in VEGF expression after radiofrequency ablation could be related to greater coagulative necrosis and, hence, a larger proportion of viable cells in the nodules dying off after RFA. Nevertheless, the present study shows that there is a relationship, though not a cause-and-effect one. Further studies to confirm the cause-and-effect relationship through molecular or functional analyses of the particular anti-angiogenic effect of RFA are lacking. It could be hypothesised that the reduction in VEGF expression after RFA is more related to the overall reduction in the volume of viable nodules (20).

Meanwhile, RFA causes less damage to surrounding normal thyroid tissue, thereby avoiding compensatory increases in VEGF during repair of normal tissue. Thus, it blocks the nutritional support for nodule regeneration from the source (21, 22). In contrast, although MWA can also reduce VEGF expression, due to the possible non-uniformity of local necrosis, the remaining small number of active cells can still maintain a certain level of VEGF expression, resulting in weaker angiogenesis inhibition than RFA. In our cohort, the duration of post-procedural pain, defined as the time until the VAS score fell below 3 without regular analgesic use, was shorter after RFA than after MWA (mean 3.2 vs 5.6 days), further supporting the clinical tolerability of RFA.

This is not only due to the minimally invasive advantages of RFA technology (accurate puncture path and small thermal damage range) but also closely related to intraoperative nursing cooperation (such as accurately establishing a fluid barrier: injecting 8–15 mL of normal saline into the space between the thyroid and trachea, and the common ca-

rotid artery to avoid thermal injury to the recurrent laryngeal nerve and large blood vessels) (23). In addition, to address the potential postoperative thyroid dysfunction, the nursing team further reduces the risk of drug-related ARs through basic medication guidance, providing additional safety assurance for RFA treatment (24). However, our limitation is that we can only measure serum inflammatory markers CRP, IL-6, and TNF- α levels at baseline and post-operative day 1. This is because although it shows the initial response, it doesn't show the resolution phase, which can be seen if measurements are taken a week or a month after treatment.

Conclusion

Thus, in conclusion, ultrasound-guided radiofrequency ablation was found to have better overall efficacy than microwave ablation for benign thyroid nodules, with greater reduction in nodule volume, higher response rate, lower acute inflammatory response, and greater reduction in VEGF expression, while maintaining thyroid and coagulation functions within normal limits. Both procedures were safe and minimally invasive in nature; however, radiofrequency ablation showed lower adverse effects and quicker onset of pain relief, making this procedure a highly attractive option for patients seeking this organ-preserving option as an alternative to surgical intervention.

Informed consent

The authors affirm that the human research participants provided informed consent for the publication of the images in Figure(s) 1A, 1B, and 1C. The participant has consented to the submission of the case report to the journal. Patients provided informed consent to publish their data and photographs.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

References

1. Boers T, Braak SJ, Rikken NET, Versluis M, Manohar S. Ultrasound imaging in thyroid nodule diagnosis, therapy, and follow-up: Current status and future trends. *J Clin Ultrasound* 2023; 51(6): 1087–100. doi:10.1002/jcu.23430
2. Grani G, Sponziello M, Filetti S, Durante C. Thyroid nodules: diagnosis and management. *Nat Rev Endocrinol* 2024; 20(12): 715–28. doi:10.1038/s41574-024-01025-4

3. Austerlitz J, Mann DS, Noel JE, Orloff LA. Thyroid nodule rupture following radiofrequency ablation for benign thyroid nodules. *JAMA Otolaryngol Head Neck Surg* 2024; 150(8): 651–7. doi:10.1001/jamaoto.2024.1400
4. Garber JR, Frasoldati A, Patkar V, Papini E. Editorial: thyroid nodule evaluation: current, evolving, and emerging tools. *Front Endocrinol (Lausanne)* 2023; 14: 1276323. doi:10.3389/fendo.2023.1276323
5. Nygaard B, Hegedüs L, Nielsen KG, Ulriksen P, Hansen JM. Long-term effect of radioactive iodine on thyroid function and size in patients with solitary autonomously functioning toxic thyroid nodules. *Clinical Endocrinology* 1999; 50(2): 197–202. <https://doi.org/10.1046/j.1365-2265.1999.00635.x>
6. Eskandar E, Kumar H, Boini A, Velasquez Botero F, El Hunjul GN, Nieto Salazar MA, Quinonez J, Dinh B, Mouhanna JE. The Role of Radiofrequency Ablation in the Treatment of Trigeminal Neuralgia: A Narrative Review. *Cureus* 2023; 15(3): e36193. <https://doi.org/10.7759/cureus.36193>
7. Noel JE, Sinclair CF. Radiofrequency ablation for benign thyroid nodules. *J Clin Endocrinol Metab* 2023; 109(1): e12–e17. doi:10.1210/clinem/dgad357
8. Knavel EM, Brace CL. Tumor ablation: common modalities and general practices. *Techniques in vascular and interventional radiology* 2013 Dec 1; 16(4): 192–200. doi: 10.1053/j.tvir.2013.08.002.
9. Boers T, Braak SJ, Brink WM, Versluis M, Manohar S. 3D ultrasound guidance for radiofrequency ablation in an anthropomorphic thyroid nodule phantom. *Eur Radiol Exp* 2024; 8(1): 115. doi:10.1186/s41747-024-00513-6
10. Thomas J, Ledger GA, Haertling T. Effects of ultrasound-guided laser ablation therapy on symptomatic benign thyroid nodules, using echolaser - results of a pilot study in the United States. *Endocr Pract* 2023; 29(12): 942–7. doi:10.1016/j.eprac.2023.08.015
11. Crespo Vallejo E, Hermosin A, Gargallo M, Villalba Á, Daguier E, Flores J, et al. Multiple overlapping microwave ablation in benign thyroid nodule: a single-center 24-month study. *Eur Thyroid J* 2023; 12(1): e220175. doi:10.1530/ETJ-22-0175
12. Tang J, Wang L, Sun Z, Liu X, Li H, Ma J, et al. Publications on ultrasound-guided thermal ablation for thyroid nodules from 2000 to 2022: a bibliometric analysis. *Int J Hyperthermia* 2022; 40(1): 2268874. doi:10.1080/02656736.2023.2268874
13. Yoo MR, Gweon HM, Park AY, Cho KE, Kim JA, Youk JH, Son EJ. Repeat Diagnoses of Bethesda Category III Thyroid Nodules: What To Do Next? *PloS one* 2015; 10(6): e0130138. doi.org/10.1371/journal.pone.0130138
14. Turkdogan S, Pusztaszeri M, Forest V I, Hier M P, Payne RJ. Are Bethesda III Thyroid Nodules More Aggressive than Bethesda IV Thyroid Nodules When Found to Be Malignant? *Cancers* 2020; 12(9): 2563. <https://doi.org/10.3390/cancers12092563>
15. Garg S, Naik LP, Kothari KS, Fernandes GC, Agnihotri MA, Gokhale JC. Evaluation of thyroid nodules classified as Bethesda category III on FNAC. *Journal of Cytology* 2017; 34(1): 5–9. <https://doi.org/10.4103/0970-9371.197590>
16. Kim NE, Raghunathan RS, Hughes EG, Longstaff XR, Tseng CH, Li S, Cheung DS, Gofnung YA, Famini P, Wu JX, Yeh MW. Bethesda III and IV thyroid nodules managed nonoperatively after molecular testing with Afirma GSC or Thyroseq v3. *The Journal of Clinical Endocrinology & Metabolism* 2023 Sep; 108(9): e698–703.
17. Chan WK, Sun JH, Liou MJ, Hsu CJ, Lu YL, Chou WY, et al. Novel and advanced ultrasound techniques for thyroid thermal ablation. *Endocrinol Metab (Seoul)* 2024; 39(1): 40–6. doi:10.3803/EnM.2024.1917
18. Boers T, Brink W, Bianchi L, Saccomandi P, van Hespden J, Wennemars G, et al. An anthropomorphic thyroid phantom for ultrasound-guided radiofrequency ablation of nodules. *Med Phys* 2024; 51(2): 826–38. doi:10.1002/mp.16906
19. Qian Y, Li Z, Fan C, Huang Y. Comparison of ultrasound-guided microwave ablation, laser ablation, and radiofrequency ablation for the treatment of elderly patients with benign thyroid nodules: A meta-analysis. *Exp Gerontol* 2024; 191: 112425. doi:10.1016/j.exger.2024.112425.
20. Kim JH, Baek JH, Lim HK, et al. Changes in thyroid volume, nodule volume, and thyroid function after radiofrequency ablation of benign thyroid nodules: a 5-year follow-up study. *Korean J Radiol* 2015; 16(4): 817–23. doi:10.3348/kjr.2015.16.4.817.
21. Oung C, Tesoro R, Marti V, Mavromati M, Lahoud MJ. Hypnosis in high-intensity focused ultrasound for thyroid nodule ablation. *Am J Case Rep* 2023; 24: e941524. doi:10.12659/AJCR.941524
22. Wang HC, Wu CW, Huang TY. Hyperthyroidism after radiofrequency ablation for thyroid nodule. *Kaohsiung J Med Sci* 2024; 40(4): 415–6. doi:10.1002/kjm2.12809
23. Motaghd Z, Chegeni H, Mosadeghkah A, Azimi Aval M, Gerami R, Ebrahimi H. Effect of ultrasound parameters of benign thyroid nodules on radiofrequency ablation efficacy. *BMC Med Imaging* 2023; 23(1): 85. doi:10.1186/s12880-023-01044-z
24. Bojunga J, Trimboli P. Thyroid ultrasound and its ancillary techniques. *Rev Endocr Metab Disord* 2024; 25(1): 161–73. doi:10.1007/s11154-023-09841-1