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Five-year results of radiofrequency and laser ablation of benign thyroid nodules: a multicenter study from the Italian minimally-invasive treatments of the thyroid group

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Abstract:	Background: Radiofrequency and laser ablation (RFA and LA) are effective treatments for benign thyroid nodules. Due to their relatively recent introduction into clinical practice, there are limited long-term follow-up studies. This study aimed to evaluate technique efficacy, rate of regrowth and retreatment over 5 years following RFA or LA and to identify predictive factors of outcome. Methods: In this multicenter retrospective study, the rate of technique efficacy, regrowth, and retreatment were evaluated in 406 patients treated with either RFA or LA and followed for 5 years after initial treatment. Propensity score matching was used to compare treatments. Cumulative incidence studies with hazard models were used to describe regrowth and retreatment trends and to identify prognostic factors. Logistic regression models and receiver operating characteristics analyses were used for risk factors and their cut-offs. Results: RFA and LA significantly reduced benign thyroid nodule volume, and this reduction was generally maintained for 5 years. Technique efficacy (defined as a reduction ≥ 50% after 1 year from the treatment) was achieved in 74% of patients (85% in the RFA and 63% in the LA group). Regrowth occurred in 28% of patients (20% in the RFA and 38% in the LA group). In the majority of cases, further treatment was not required as only 18% of patients were retreated (12% in the RFA and 24% in the LA group). These data were confirmed by propensity score matching. Cumulative incidence studies showed that RFA was associated with a lower risk of regrowth and a lower risk of requiring retreatment over time. Overall, technique inefficacy and regrowth were associated with low energy delivery. Retreatments were more frequent in young patients, in large nodules, in patients with lower volume reduction at 1-year, and in cases of low energy delivery (optimal cut-off was 918 J/mL for RFA). Conclusions: Both thermal ablation techniques result in a clinically significant and long-lasting volume reduction of benign thyroid nodules. The risk of regrowth and needing retreatment was lower after RFA. The need for retreatment was associated with young age, large baseline volume, and treatment with low energy delivery.

POINT-BY-POINT RESPONSE to THY-2020-0202.R1 decision letter

First of all, we would like to thank you for the changes to the manuscript and for your valuable feedback. Please find the point-by-point response to all your comments. Changes in the manuscript have been highlighted in blue ink. We hope to have answered all your questions/comments satisfactorily.

Main text

Line 91: As you suggested, we have left "in Italy".
Line 114-115: We have stated "follow-up of at least 5 consecutive years after the first ablation" and we have removed "*Patients fitting the criteria (i) and (ii), who had been treated before 2015, but whose follow-up was interrupted because they underwent surgery were also included. By contrast, all the patients treated from 2015 onwards were excluded (as they could not complete a 5-year follow-up by the time of data analysis)*".
Lines 124: We have added "status". The way we assessed thyroid function status is specified on lines 132-133.
Line 133: We have added the reference suggested by the peer Reviewer.
Line 133: We have specified that laboratory examinations included TSH, FT3, and FT4.
Lines 187-311: Throughout the "Results" section we have renumbered all the Tables and Figures.
Lines 218: We have specified that patients who "lost technique efficacy" were defined as patients where nodule "regrowth diminished VRR to less than 50%" during the follow-up.
Lines 247-250: Given that Figure 5 has been removed we have reported the rates of technique efficacy, regrowth, and retreatments in the RFA and LA groups after propensity score matching.
Line 389: As you suggested, we have removed "It has to be taken into account that although thermal ablations should not affect substantially pathology results in case of a thyroid carcinoma (42), spots of capsular/vascular invasion as well as microcarcinomas within the ablated area might be no longer found (43)".

Figure Legends and Figures

Line 12: we have specified that PEI is for percutaneous ethanol injection
Lines 2-32: Figures have been renumbered and changed as requested.

Tables

Table 1 and Table 3 have been changed to Supplementary Tables 1-2 and the remaining Tables have been renumbered accordingly.
In the renumbered Table 1, we have removed the symbol "§", we have defined that comparison was to baseline for all years, and we have defined that MITT is for minimally invasive treatments of the thyroid.

Five-year results of radiofrequency and laser ablation of benign thyroid nodules: a multicenter study from the Italian minimally-invasive treatments of the thyroid group

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Running title: **5-year outcomes of thermal ablations**

Key words: 5 years, follow-up, RFA, laser, benign thyroid nodules, regrowth, retreatment, cumulative incidence

Abstract

Background: Radiofrequency and laser ablation (RFA and LA) are effective treatments for benign thyroid nodules. Due to their relatively recent introduction into clinical practice, there are limited long-term follow-up studies. This study aimed to evaluate technique efficacy, rate of regrowth and retreatment over 5 years following RFA or LA and to identify predictive factors of outcome.

Methods: In this multicenter retrospective study, the rate of technique efficacy, regrowth, and retreatment were evaluated in 406 patients treated with either RFA or LA and followed for 5 years after initial treatment. Propensity score matching was used to compare treatments. Cumulative incidence studies with hazard models were used to describe regrowth and retreatment trends and to identify prognostic factors. Logistic regression models and receiver operating characteristics analyses were used for risk factors and their cut-offs.

Results: RFA and LA significantly reduced benign thyroid nodule volume, and this reduction was generally maintained for 5 years. Technique efficacy (defined as a reduction ≥ 50% after 1 year from the treatment) was achieved in 74% of patients (85% in the RFA and 63% in the LA group). Regrowth occurred in 28% of patients (20% in the RFA and 38% in the LA group). In the majority of cases, further treatment was not required as only 18% of patients were retreated (12% in the RFA and 24% in the LA group). These data were confirmed by propensity score matching. Cumulative incidence studies showed that RFA was associated with a lower risk of regrowth and a lower risk of requiring retreatment over time. Overall, technique inefficacy and regrowth were associated with low energy delivery. Retreatments were more frequent in young patients, in large nodules, in patients with lower volume reduction at 1-year, and in cases of low energy delivery (optimal cut-off was 918 J/mL for RFA).

Conclusions: Both thermal ablation techniques result in a clinically significant and long-lasting volume reduction of benign thyroid nodules. The risk of regrowth and needing retreatment was lower after RFA. The need for retreatment was associated with young age, large baseline volume, and treatment with low energy delivery.

Introduction

Benign thyroid nodules are a common clinical finding. Although most of them are asymptomatic, in a small percentage of patients (10-15%) they increase over time, causing local symptoms and cosmetic concerns (1). In these cases, the conventional remedy is thyroid surgery. Recently, however, minimally invasive non-surgical treatments, mostly image-guided thermal ablations, such as laser and radiofrequency ablation (LA and RFA), have emerged as an alternative approach to treat symptomatic benign thyroid nodules (2-4).

Both RFA and LA are outpatient procedures, which are generally performed under local anesthesia. Technically, either an electrode-needle generating an alternating electric field (in case of RFA) or one or more optical fibers conveying laser light (in case of LA), are inserted into the nodule under ultrasound (US) guidance, to induce rapid heating and destruction of the target zone. Treatment is accompanied by the formation of coagulative necrosis, and, over time, by fibrotic changes and progressive nodule shrinkage. RFA is generally performed with the moving shot technique, whereby the tip of the electrode is sequentially moved from the medial and deepest part of the nodule to its most superficial and lateral parts. By contrast, LA requires the positioning of one or more optical fibers into the target nodule, which might be eventually pulled back in case of larger nodules (1). The mean costs of RFA and LA are similar in Italy, a fixed charge for RFA (i.e., one device per nodule, whose cost ranges from $500-1000) and a variable one for LA depending on the number of fibers (the larger the nodule volume to be treated the higher the cost as more fibers are needed, one fiber costs $300-500) (5).

The use of RFA and LA to treat symptomatic benign thyroid nodules is supported by robust evidence of efficacy and tolerability. Both treatments demonstrated a significant reduction of thyroid nodule volume (6-8), associated with significant improvement of local symptoms. These procedures are reported as well tolerated in large retrospective series, with a risk of major complications (recurrent laryngeal nerve injury or damage to cervical structures) lower than 1% (9, 10). Unfortunately, there are limited number of long-term follow-up studies evaluating not only volume reduction and technique efficacy, but also assessing regrowth and retreatment rates. Thus, this multicenter retrospective study aimed (*i*) to evaluate the rate of technique efficacy, regrowth, and retreatment following thyroid nodule thermal ablations, as well as (*ii*) to identify potential risk factors and cut-off values predicting efficacy, regrowth, and retreatment using regression models and receiver operating characteristics (ROC) analyses.

Materials and Methods

Study design

This is a retrospective multicenter study, whose primary outcome was to describe the rate of technique efficacy, regrowth, and retreatment 5 years after ablation of a benign thyroid nodule. Secondary outcomes were the identification of predictive variables of efficacy, regrowth, and retreatment. Inclusion criteria of patients were: (*i*) benign cytology prior to ablation (diagnostic category Thy2/Tir2 or Bethesda II, (11, 12), as assessed by fine needle aspiration biopsy (FNAB) and cytologic examination; (*ii*) no prior thyroid treatment (radioiodine, ethanol injection); (*iii*) follow-up of at least 5 years after the first ablation; and (*iv*) patient consent to use their data for this study. This study was conducted in accordance with the declaration of Helsinki, and the protocol for this retrospective analysis was approved by the Institutional Review Board (268_2019 FYTNAB).

The study finding was presented during the 2nd meeting of the Minimally-invasive treatments of the thyroid (MITT) Group (4, 13), held in Milan in February 2019. The Italian centers belonging to the MITT group were invited with an open call to contribute patient data. Centers were invited to contribute with data of consecutive annual cohorts of patients with benign thyroid nodules treated before 2015 (**Table 1**). The following parameters were collected: age, sex, year of treatment, type of procedure, energy delivered (J), baseline nodule volume (mL), nodule structure, nodule function status, nodule volume after 1, 2, 3, 4, 5 years from the procedure (mL), symptom relapse, type of retreatment, final pathology (in case of surgery), nodule volume after a second thermal ablation (mL). In case of LA, the number of fibers was recorded, and the type of RFA electrode was recorded. Nodule volume was measured by ultrasound examination. Ultrasound scans were generally performed with linear transducers except for very large nodules, whose volume was quantified with convex transducers. For thyroid nodule volume (V) determinations, the following formula was used: $V = \pi abc/6$ (where V is the volume, a is the maximum diameter, and b and c are the other two perpendicular diameters). Energy delivered was expressed as J/mL. Joules (or kilocalories) were either recorded from the machine or calculated as Watt * s (14). Nodule function status was assessed with laboratory examinations (TSH, FT3, and FT4) and, in case TSH was < 0.4 microU/mL, with thyroid scintigraphy (2, 15).

Definitions

Nodule structure was classified as solid if the fluid component was ≤10%, predominantly solid if the fluid component was between 11-50%, predominantly cystic if the fluid component was between 51-90%, and cystic if the fluid component was >90% (16). ***Volume reduction ratio*** (VRR) was defined as the percentage reduction in volume and it was calculated as follows: VRR = [(initial volume – final volume)/initial volume] x 100. Given that our cohort included some patients who were retreated, in order to analyze nodule volume reduction after the first procedure/ablation, data after retreatments were excluded. ***Technique efficacy*** was defined as a volume reduction ≥50% after 1 year from the treatment (16, 17). ***Regrowth*** was defined as a ≥50% increase compared to the previous smallest volume at US examination (16, 18).

Statistical analyses.

All statistical analyses were carried out in R system for statistical computing (Ver. 5.0; R Development Core Team, 2018). Statistical significance was set at $p<0.05$. Shapiro-Wilk test was used for quantitative (continuous) variables to check for distribution normality. Continuous variables are reported as median with range (minimum-maximum). Categorical variables are reported as absolute frequencies and/or percentages (rates of technique efficacy, regrowth and retreatment). Continuous variables were compared using the Mann–Whitney test (and Kruskall Wallis test), depending on data distribution and number of groups. Categorical variables were compared using the Chi-square test or Fischer's exact test whenever appropriate. Variations over time of nodules' volume were evaluated with non-linear mixed-effects models (NLME) for repeated measures. Multiple comparisons of nodules' volume respect to different follow-up periods (baseline vs 1, 2, 3, 4, and 5 years) were performed with Friedman test for repeated measures and p-values adjusted with Bonferroni post-hoc test.

To compare the patients treated with RFA to those treated with LA, in order to control for potential confounders and selection bias, we performed a sensitivity analysis using propensity score matching with the R package 'MatchIt' (method nearest neighbor). The patients were matched 1:1 by age, sex, thyroid nodule volume, nodule structure (solid), and thyroid function.

To describe regrowth trends we used the cumulative incidence function (CIF), which takes into account the presence of competing risks (such as retreatment in our case). Then, cumulative incidence of regrowth in RFA and LA groups was compared with the Gray test. To identify significant predictors of regrowth over time (hazard ratio (HR) with 95% confidence interval) we used the Fine and Gray competing risk regression model (19). CIF and CRR analyses were performed with the R package *cmprsk* (20). To describe the likelihood of not being retreated we used the standard Kaplan-Meier method. Cox proportional hazard regression model was implemented to identify predictors of retreatment and to estimate HR with 95% CI.

To identify potential risk factors for technique inefficacy, regrowth, and retreatment, we conducted a univariate logistic regression analysis and calculated the odds ratios of age, sex, baseline volume, nodule structure and function, 1-year nodule reduction, technique efficacy and regrowth for the outcome technique inefficacy, regrowth, and retreatment. Statistically significant variables at a p value of < 0.10 level on univariate analysis were selected as candidate prognostic factors for multivariate logistic regression analyses. Energy/volume and technique could not be tested simultaneously for collinearity. So, we decided to evaluate the association between energy delivered and outcome of thermal ablations.

Receiver operating characteristic (ROC) analyses were used to calculate the accuracy of volume, 1-year volume reduction, and energy, as predictors of technique efficacy, regrowth, and retreatment. Area under the (ROC) curves with 95% confidence interval, were interpreted according to Sweets criteria, and were used to identify a cut-off value of baseline volume, 1-year nodule volume and energy delivered that best predicted technique efficacy, regrowth, and retreatment. Specificity and sensitivity were also calculated (95% confidence interval, CI). The best possible cut-off point was defined as the highest Youden Index [(specificity + sensitivity) – 1 (R package 'OptimalCutPoints')]. DeLong method was used to test the statistical significance of the difference between the areas under the curve.

Results

Study cohort and characteristics.

Eight centers participated in this retrospective study (Genova, Latina, Lecce, Milano, Napoli, Teramo, Torino, Trieste). Each center provided data of consecutive annual cohorts of all patients with benign thyroid nodules treated in the years reported (**Supplementary Table 1).** Data from 477 patients with benign thyroid nodules were collected. Among these patients, 59 patients were lost during the follow-up and 12 patients did not meet criteria *i* and *ii* and were excluded. Inclusion criteria were met by 406/477 patients (85%), who were selected for this study (**Supplementary Table 1**).

Median age of the study cohort was 57 years (17-87); there were 304 female (75%) and 102 male (25%). Among the 406 patients selected for this study, 216 patients (53%) were treated with RFA, while 190 patients (47%) were treated with LA. Treatment with LA was performed between 2009 and 2014, consistent with the fact that LA is the first thermal ablation technique that was introduced in clinical practice to treat thyroid nodules (21), while RFA was more recent (22). LA was performed with 1-3 optical fibers and a 1064 nm diode laser source (21, 23). The number of fibers depended on nodule volume and morphology. Treatments with RFA were performed between 2012 and 2014. RFA was performed with the moving shot technique and a monopolar 18-G needle (22, 24).

Nodule volume reduction and technique efficacy

A total of 75% of patients had a solid nodule, 19% had a predominantly solid nodule, 5% had a predominantly cystic nodule, and 1% had a cystic nodule. Nodules were non-functioning in 91% of patients. The median baseline nodule volume was 14.3 mL (0.4-179.0); 17.2 mL (0.4-179.0) in the RFA group and 12.2 mL (1.7-86.0) in the LA group (**Table 1**). Nodule volume was significantly reduced by the first ablation (**Table 1**), $p<0.001$ for repeated measures. Median thyroid nodule VRR were 63%, 67%, 68%, 69%, and 70% at 1, 2, 3, 4, and 5 years after the first ablation, respectively. In all the patients treated with RFA (n=216), median thyroid nodule VRR were 72%, 75%, 76%, 76%, and 77% at 1, 2, 3, 4, and 5 years after the ablation, respectively. In all the patients treated with LA (n=190), median VRR were 55%, 58%, 59%, 57%, and 57%, at 1, 2, 3, 4, and 5 years after the ablation, respectively (**Table 1**). The technique efficacy was 74%; 85% for RFA (183/216) and 63% for LA (119/190), *p*<0.001.

Regrowth and retreatment rates

A total of 28% of patients (115/406) had thyroid nodule regrowth. Among the 115 patients with regrowth, 69% of patients (79/115) lost technique efficacy (the regrowth diminished the VRR to less than 50%), 26% of patients (30/115) had symptom relapse, and 28% of patients (32/115) were retreated. Regrowth was observed in 20% of patients treated with RFA (43/216) and in 38% of patients treated with LA (72/190), *p*<0.001. Consistent with the efficacy of both procedures and the lower tendency for regrowth after RFA, we found a good correlation between 1-year and 5-year VRR after both treatments, even if it was more pronounced after RFA (**Figure 1**). **Figure 2A-B** shows the non-cumulative and cumulative regrowth rates over the 5 years of follow-up.

The vast majority of patients (82%) did not receive any further treatment after the first thyroid ablation, while 18% (72/406) underwent a second procedure. In particular, in the RFA group 12% of patients (26/216) were retreated, while in the LA group 24% of patients (46/190) were retreated (*p*<0.001). In terms of type of retreatment, 43/406 patients had a thyroidectomy (11%), 13 patients (3%) underwent a second LA, 10 patients (2%) underwent a second RFA, 2 patients (0.5%) were treated with radioiodine, 2 patients underwent a second RFA and thyroidectomy, while 1 patient (0.25%) underwent ethanol injection, and 1 patient underwent a second LA and thyroidectomy (**Figure 2C**). Patients who underwent a second ablation had a median nodule volume of 12.5 (3.0-114.0) mL before the retreatment, which was reduced to 6.8 (1.5-40.8) mL after 1 year from the retreatment, with a median volume reduction of 44%. **Figure 2D** shows nodule volume reductions of every patient at follow up after retreatment.

Comparison between RFA and LA with propensity score matching analysis

After propensity score matching analysis, we selected 76 patients treated with RFA and 76 patients treated with LA, who did not differ in terms of age, sex, baseline nodule volume, nodule structure, nodule function (**Supplementary Table 2**). It was impossible to match the two groups in terms of delivered energy, because LA is associated with a significantly lower amount of energy delivery. **Figure 3A-D** shows the VRR after RFA and LA (*p*<0.001 for technique), before and after propensity score matching. Both procedures significantly reduced nodule volume (*p*<0.001 vs baseline), but nodule volume reduction after RFA was greater than after LA (*p*=0.02) (**Figure 3E**). After propensity score matching, the VRR after the first ablation was 72%, 74%, 75%, 75%, and 75% at 1, 2, 3, 4, and 5 years, respectively, in patients treated with RFA (=76). In patients treated with LA (=76), the VRR was 54%, 57%, 55%, 55%, and 56%, at 1, 2, 3, 4, and 5 years, respectively, after the first ablation. RFA was associated with a greater rate of technique efficacy, 82% in the RFA vs 66% in the LA group (*p*=0.001), with a significantly lower percentage of regrowth, 17% in the RFA vs 34% in the LA group (*p*=0.02), and a significantly lower percentage of retreatments, 14% in the RFA vs 32% in the LA group (*p*=0.01). Also, after propensity score matching there was a good correlation between 1-year and 5–year volume reduction, which was more pronounced after RFA (ρ=0.79, *p*<0.001) than LA (ρ=0.69 *p*<0.001).

Cumulative incidence of regrowth and retreatment

Given that regrowth and retreatment are time-dependent events, we assessed their cumulative incidence over time. When looking at regrowth, we calculated the cumulative incidence of regrowth in the presence of retreatment as a competing risk (i.e. an event precluding the occurrence of regrowth). The estimated cumulative incidence rates of regrowth in the entire patient cohort are shown in **Figure 4A-B**. The Fine and Gray competing risk regression model showed that energy delivered was the only parameter that was independently associated with the risk of regrowth (**Table 2**). When looking at the cumulative incidence of regrowth (and retreatment as the competing event) for type of treatment, we found that RFA was associated with a significantly lower risk of regrowth as compared to LA (*p*<0.001 Gray Test), while there were no differences in terms of retreatment (*p*=0.08), shown in **Figure 4C**. Similar results were obtained also after propensity score matching (**Figure 4D**). When looking exclusively at retreatments, there was a significant lower risk of being retreated for RFA than LA (after propensity score matching, $p<0.01$) (**Figure 4E-F**). Multivariate Cox model showed that young age, larger baseline volume, lower energy delivery, lower technique efficacy, and regrowth were all significantly associated with the risk of being retreated (**Table 2**).

Risk factors of technique inefficacy, regrowth, and retreatment

On logistic regression model analyses, a lower amount of energy delivered per mL of tissue was the only parameter that was significantly associated with technique inefficacy and regrowth (**Supplementary Table 3**). Younger age, larger baseline volume, lower amount of energy, technique inefficacy, and regrowth were all significantly and independently associated with the likelihood of being retreated (**Supplementary Table 3**).

On ROC curves analysis, we found that energy delivered had an AUC of 0.65 (0.59, 0.72) and the cut-off value best predicting technique efficacy was 566 J/mL (sensitivity =0.72; specificity =0.56). After technique stratification, only the energy delivered by RFA had a moderate accuracy to predict technique efficacy with an AUC of 0.72 (0.60, 0.83) and a cut-off value of 1360 J/mL ($p=0.01$). When looking at retreatments, baseline volume had an AUC of 0.63 (0.56, 0.70); it was 0.68 (0.57, 0.79) in the RFA group and 0.67 (0.58, 0.76) in the LA group. Baseline volume cut-offs predicting retreatment were 22.1 mL for RFA and 14.5 mL for LA. The 1-year VRR to predict retreatment had an AUC of 0.79 (0.74, 0.85) and a cut-off of 58%. After technique stratification, the 1-year VRR after RFA had an AUC of 0.82 (0.73, 0.91) and a cut-off best predicting retreatment of 66%. The 1-year VRR after LA had an AUC of 0.74 (0.66, 0.88) and a cut-off best predicting retreatment of 54%. The delivered energy had an AUC of 0.70 and its cut-off value best predicting retreatment was 556 J/mL (sensitivity =0.82; specificity =0.55). After technique stratification, only the energy delivered by RFA had a good accuracy to predict retreatment, with an AUC of 0.83 (0.75, 0.92) and a cut-off value of 918 J/mL ($p<0.001$).

Non-benign pathology

A total of 46/406 patients (11%) were operated on during follow-up. Final histologic diagnosis showed benign nodules in 27/46 patients (59%), non-benign pathology in 16/46 patients (35%), while in 3 patients final pathology results went missing (6%). Non-benign pathology included: 6 incidental microcarcinomas outside the ablated nodule, 4 follicular carcinomas, 3 papillary carcinomas, 3 follicular tumors of uncertain malignant potential. When looking at the entire patient cohort, non-benign pathology was found in 16/406 patients (3.9%), excluding microcarcinomas it was found in 10/406 (2.4%). In all centers except one, patients underwent 2 FNAB for cytology assessment before the procedure. There were no differences in the rate of non-benign pathology among the patients who underwent one FNAB (4/103) and two FNAB (12/303) (p=0.99).

Based on analysis of odds ratios of malignancy for age, sex, baseline volume, 1-year volume reduction, nodule structure, success, regrowth, and energy delivered, only male sex was associated with a greater risk of malignancy (**Supplementary Table 4**).

In the 16 cases with non-benign pathology, we noticed that most patients had been retreated after 1 year, and the only aspect that could be compared to the other nodules was the 1-year VRR. We analyzed the median 1-year VRR of patients who did not require further treatments (n=334), the median 1-year VRR of patients who were operated on and were found having a benign nodule (n=27), and of patients who were found having a non-benign pathology (n=16). The 1-year VRR was 67%, 46%, and 27%, respectively (p<0.001 for all groups). ROC curve analysis showed that the 1-year VRR had an AUC of 0.823 (95% CI) and its cut-off value best predicting non-benign pathology was 21% (sensitivity = 50%; specificity = 98%), according to the maximum of the Youden Index. When excluding microcarcinomas, the 1-year VRR had a AUC of 0.853 (95% CI) and the cut-off value best predicting malignancy was still 21% (sensitivity =50%; specificity = 98%).

Discussion

Many investigators have demonstrated that US-guided thermal ablation is a safe and clinically effective procedure for the treatment of benign thyroid nodules that become symptomatic. Only few studies with extended follow-up (5 years or more), however, have evaluated the long-term efficacy (regrowth and need of retreatment) (18, 25-27). Thus, we conducted this first multicenter study enrolling patients who were followed for five years after a single session of RFA and/or LA.

Consistent with previous reports, a single session of RFA or LA significantly reduced thyroid nodule volume and this was maintained in most patients during a five-year period (28). In the current study, VRR after RFA was lower than in previous studies (26) that reported a 89% and 90% nodule volume decrease at 1 and 3 years, respectively. In these previous studies, only part of the patients completed 5-year follow-up (follow-up range was 36-81 months) and, most importantly, they were treated on average twice (mean number of session was 2.2 ± 1.4) (26). Conversely, our VRR after RFA are similar to those reported by Sim (18), who found a volume reduction of 77%, and by Deandrea (27), who found a volume reduction of 70% after the first RFA session. The VRR we found for LA is consistent with studies by Papini (8) and Dossing (25).

Technique efficacy was achieved in 74% of patients and was significantly associated with the delivered energy. The energy cut-off best predicting technique efficacy was 566 J/mL. Although it had a poor accuracy, this cut-off is consistent with previous data by Gambelunghe (29) and De Freitas (30). The accuracy of the energy cut-off increased after technique stratification only for RFA, where energy cut-off was 1360 J/mL. Propensity score matching showed that technique efficacy was achieved more frequently in patients treated with RFA (82%) than in those treated with LA (66%) possibly because RFA was associated with a greater amount of energy delivered. This variability could be due to the different modalities of action of RFA and LA, which are not only two operator-dependent techniques, but they have also specific modalities of production and distribution of thermal energy (24, 31). For example, laser technology directs high-frequency energy to a well-delimited area of tissue, heat deposition is greatest near the thermal source with a rapid energy/heat decay in the surrounding tissue (32). When performing RFA with monopolar electrodes, the ones that were used in this study, the patient is part of a closed-loop circuit that includes the radiofrequency generator, the electrode needle, and a large dispersive electrode (ground pads), such that heat is distributed in a larger area of surrounding tissue (33).

The direct comparison of the two techniques (RFA, LA) was assessed in recent studies reaching differing conclusions (28, 31, 34-36). Our results are consistent with the conclusions of two metanalyses and the only randomized controlled trial comparing these techniques. Ha et al. reported that RFA was more effective than LA in terms of volume reduction after 6 months from the procedure (77.8% vs 49.5% after RFA and LA, respectively) (34). Trimboli et al. similarly reported that volume reduction after 1, 2, and 3 years was 68%, 75%, and 87% with RFA as compared to 48%, 52%, and 45% with LA, respectively (28). Finally, in the only randomized controlled trial comparing these two treatment modalities, technique efficacy was achieved in 87% of patients treated with RFA as compared to 67% of patients treated with LA (36) and RFA was associated with a significantly greater nodule volume reduction after 6 months (64% vs 53%) (36). These data appear consistent with our results.

Nodule regrowth occurred in 28% of patients. Cumulative regrowth rate increased progressively over time. Nodule regrowth was not clinical significant as symptom recurrence occurred in 26% of cases of regrowth, and a second treatment was performed in 28% of patients whose nodule regrew. Our results are similar to those of Sim and colleagues who reported a regrowth in 24% of the nodules, mostly after 2-4 years of follow-up. Nevertheless, it is difficult to compare our data to those of other investigators, due to the different definitions used (8, 37) and the significant loss of patients to follow-up in previous studies (18, 26, 27). Although we found a good correlation between 1-year and 5-year volume reduction, odds ratio assessment demonstrated that the only variable significantly associated with nodule regrowth after ablation was the quantity of energy delivered. However, given that energy was a poorly accurate predictor of regrowth, our findings suggest that nodule regrowth may be associated, not only to energy delivery (14, 30), but also to the type of technique (38), as RFA resulted in a significantly lower regrowth rate (17%) as compared to LA (34%). Consistent with these rates, cumulative risk curves showed that RFA had a significantly lower risk of regrowth over time. One of the reasons accounting for this difference could be that RFA is performed by sequentially moving the tip of the electrode across the entire nodule area, which allows the tailoring of the procedure to the variable features of the nodules, maximizing the ablation of the marginal areas of the lesion. The undertreatment of nodule margins (38) and the nodule composition (specifically, solid vs spongiform) (39), together with other minor biological characteristics (25) are additional factors that could account for nodule regrowth.

In our study, the vast majority of patients did not require multiple treatments, as only 18% of them underwent a second procedure over the 5 years of follow-up. LA was associated with a significantly higher rate of retreatments as compared to RFA (32% vs 14%, respectively). The rate of retreatments after LA is consistent with the rate reported by Dossing, which was 35% (25). Consistent with this finding, Kaplan-Meier curves showed that patients with RFA were more likely not to be retreated over time. Retreatments were more likely to happen in young patients, in larger nodules, in patients with lower 1-year VRR, and when the energy delivered was low (40).

The clinical practice data analyzed in this study provides a few relevant cut-offs for the prediction of retreatment. The baseline volume cut-off that best predicted the need of retreatment was 22 mL after RFA and 14.5 mL after LA. This is consistent with data from a few previous studies that found nodules larger than 20 mL generally require more than one treatment session (6, 26) and that in nodules larger than 20 mL the results might not be as satisfactory as thyroidectomy (41). The 1-year VRR cut-off that best predicted retreatment was a reduction <66% after RFA and a reduction <54% after LA. For energy delivered, the cut-off best predicting retreatment was 556 J/mL, and it improved in accuracy after technique stratification, changing to 918 J/mL after RFA.

Thyroid surgery represented 60% of the retreatments (46/406 patients) in this study. On histologic examination, 16/46 (35%) of these patients had non-benign pathology (3.9% of all the treated patients and 2.4% if we excluded microcarcinomas). Male sex was significantly associated with the risk of non-benign pathology and, importantly, most patients with non-benign pathology did not achieve technique efficacy and were retreated after 1 year from the first ablation. Due to the timing of surgery, we could not observe an association between regrowth and non-benign pathology. ROC analysis showed that a nodule volume decrease less than 20% after 1 year was a predictive factor of the risk of non-benign pathology. So, for patients whose nodule decrease is less than 20% after thermal ablation, a repeat cytological assessment and, possibly, surgery appear more appropriate than a repeat thermal ablation procedure.

Main limitations of the present study are its retrospective and non-randomized design, and the collection of data from different centers with possible selection bias. In addition, the procedures were performed by different operators, which has to be taken into account as thermal ablation is an operator-dependent technique. On the other hand, due to its multicenter design, our data provide a real world assessment of thermal ablation outcomes. Most importantly, this is the first follow-up study where a large series of patients has been followed for 5 years, allowing us to report cumulative risk of regrowth and retreatment over time not only for RFA but also for LA.

In conclusion, both RFA and LA induce a clinically relevant volume reduction of benign thyroid nodules that persists several years after the procedure in most patients. Technique efficacy is achieved in the vast majority of patients and was associated with the energy delivered. Regrowth occurs in less than one-third of patients but in the majority of cases did not require further treatment. Retreatments are more likely in young patients, in larger nodules, and in patients with a low 1-year volume reduction. RFA is associated with a lower risk of regrowth and retreatment as compared to LA, which may be due to the different amount of energy delivered with this technique. Finally, a VRR $\leq$20% after one year should raise suspicion of an underlying malignancy and prompt for repeat FNAB or thyroid surgery.

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Disclosure statement

FS is a consultant for HS AMICA (LT), GM is a consultant for ELESTA Srl Calenzano (FI). All the other Authors have no competing financial interest to disclose.

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Figure Legends

Figure 1. Correlation between 1-year and 5-year volume reduction

Scatter plot for entire patient cohort (**A**), LA group (**B**), and RFA group (**C**).

Figure 2. Regrowth rates, type of retreatments, and volume reductions after a second procedure. A. Non-cumulative regrowth rate. This figure describes the distribution of the first regrowth over time. In the RFA group regrowth was observed in 20% of nodules (43/216). In the LA group regrowth was observed in 38% of nodules (72/190). RFA group: 0% nodules (0/216) at 1 year; 6.1% (13/214) at 2 years; 2.5% (5/203) at 3 years; 8.1% (16/197) at 4 years; 4.7% (9/192) at 5 years. LA group: 0.5% nodules (1/190) at 1 year; 8.4% (15/179) at 2 years; 13.1% (21/160) at 3 years; 11% (17/155) at 4 years; 12.5% (18/144) at 5 years. **B.** Cumulative regrowth rate. This figure describes the distribution of nodule regrowth over time, taking into account that some nodules regrew more than once. **C**. Type of retreatment distribution (%) without the patients non-retreated. PEI is for percutaneous ethanol injection. **D**. Spaghetti plot showing single patient nodule volume reduction after a second thermal ablation (one patient with an outlayer volume has been excluded). Dotted line is for LA and solid line is for RFA.

Figure 3. Distribution of volume reduction ratio (%) 1 year after the procedure.

Distribution of VRR (%) in RFA and LA groups 1 year after the procedure in the entire patient cohort (**A**, **C**), and after propensity score matching (**B**, **D**). In both cases, VRR was significantly different in RFA and LA groups, *p*<0.001 with Chi-square Test. PSM is for propensity score matching VRR is for volume reduction ratio. E. Comparison between RFA and LA after propensity score matching. Trends of volume reduction (baseline and after 1, 2, 3, 4, and 5 years from the thermal ablation). Both procedures significantly reduced nodule volume (*p*<0.001 vs baseline), but RFA reduced nodule volume more than LA (*p*=0.02, Non Linear Mixed Effect Model).

Figure 4. Cumulative incidence of regrowth and retreatment

A-B. Cumulative incidence of regrowth and retreatment (as the competing event) with the Competing Risk method. Number at risk includes the patients who had not had a regrowth or retreatment. **C.**

Cumulative incidence of regrowth and retreatment for RFA and LA in the entire patient cohort. RFA and LA significantly differed in terms of regrowth ($p<0.001$, Gray Test) but not in terms of retreatment ($p=0.08$). **D.** Cumulative incidence of regrowth and retreatment for RFA and LA after propensity score matching. RFA and LA significantly differed in terms of regrowth ($p<0.01$, Gray Test) but not in terms of retreatment ($p=0.34$). **E-F**. Kaplan Meier curves describing no retreatment probability for RFA and LA after propensity score matching ($p=0.01$).

Table 1. Nodule volumes and nodule volume reduction

	Baseline	1 Year	2 Years	3 Years	4 Years	5 Years
ALL PATIENTS						
Nodule Volume (mL)	14.3 (0.4-179.0)	5.2* (0.0-242.0)	4.8* (0.0-214.0)	4.3* (0.0-96.0)	4.2* (0.0-88.7)	4.0* (0.0-62.0)
Volume Reduction Ratio (%)	-	63.3 (-50.0;99.7)	67.5 (-80.4;99.9)	68.3 (-63.5;1.0)	68.7 (-54.9;1.0)	70.4 (-50.0;1.0)
Number (cumulative) of patients not retreated	406	406	387	363	353	334
Number (cumulative) of patients retreated (%)	0	0	19 (4.7%)	43 (10.3%)	53 (13.1%)	72 (17.7%)
Surgery			*12*	*13*	*4*	*14*
MITT			*5*	*9*	*6*	*4*
MITT + surgery			*2*	*1*		
I-131				*1*		*1*
RFA GROUP						
Nodule Volume (mL)	17.2 (0.4-179.0)	4.9* (0.0-242.0)	4.7* (0.0-214.0)	4.4* (0.0-96.0)	4.0* (0.0-89.0)	3.9* (0.0-62.0)
Volume Reduction Ratio (%)	-	72.4 (-35.2-99.7)	74.6 (-24.9-99.9)	75.9 (-48.2; 1.0)	76.3 (-34.5; 1.0)	77.1 (-34.5; 1.0)
Number (cumulative) of patients not retreated	216	216	208	203	197	190
Number (cumulative) of patients retreated (%)	0	0	8 (3.7%)	13 (6.0%)	19 (8.8%)	26 (12.0%)
Surgery			*4*	*2*	*2*	*5*
MITT			*2*	*3*	*4*	*2*
MITT + surgery			*2*			
LA GROUP						
Nodule Volume (mL)	12.2 (1.7-86.0)	5.5* (0.3-52.0)	4.8* (0.2-39.0)	4.3* (0.2-46.8)	4.2* (0.2-39.7)	4.1* (0.1-35.0)
Volume Reduction Ratio (%)	-	54.9 (-50.0-95.7)	58.3 (-80.0-97.0)	58.8 (-63.5; 93.8)	57.5 (-54.9; 1.0)	56.7 (-50.0; 97.8)
Number (cumulative) of patients not retreated	190	190	179	160	156	144
Number (cumulative) of patients retreated (%)	0	0	11 (5.8%)	30 (15.8%)	34 (18.4%)	46 (24.2%)
Surgery			*8*	*11*	*2*	*9*
MITT			*3*	*6*	*2*	*2*
MITT + surgery				*1*		
I-131				*1*		*1*

Nodule volume and volume reduction are presented as Median (Min-Max). Nodule volume and volume reduction do not include data after retreatments. *$p<0.001$, Friedman test for repeated measures vs baseline. Surgery, MITT (minimally invasive treatments of the thyroid), MITT+surgery, and I-131 refer to the number of patients who underwent these procedures as retreatments in each year. MITT include radiofrequency ablation, laser ablation, and ethanol injection.

Table 2. Fine-Gray competing risk and Cox proportional hazard regression models

		FINE-GRAY COMPETING RISK REGRESSION MODEL			
		REGROWTH			
		Univariate CRR model		**Multivariate CRR model**	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (years)		0.98 (0.97-0.99)	0.01	0.99 (0.98-1.01)	0.60
Sex	Male	1.00 (ref)		1.00 (ref)	
	Female	1.53 (0.96-2.43)	0.09	1.41(0.84-2.36)	0.19
Baseline volume (mL)		0.99 (0.97-1.00)	0.09	0.99 (0.98-1.01)	0.36
Nodule structure	S	1.00 (ref)			
	PS	0.70 (0.40-1.19)	0.18	//	//
	PC/C	0.94 (0.45-1.93)	0.86		
Nodule function	AFTN	1.00 (ref)		1.00 (ref)	
	Non-AFTN	2.72 (1.02-7.26)	0.04	2.39 (0.30-18.93)	0.41
1-year reduction (%)		0.76 (0.36-1.60)	0.47	//	//
Energy/volume (J/mL)		0.99 (0.99-1.00)	<0.001	0.99 (0.99-1.00)	**0.001**
		COX PROPORTIONAL HAZARD REGRESSION MODEL			
		RETREATMENT			
		Univariate Cox model		**Multivariate Cox model**	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (years)		0.98 (0.96-1.00)	0.01	0.98 (0.96-0.99)	**0.01**
Sex	Male	1.00 (ref)			
	Female	1.01 (0.59-1.71)	0.99	//	//
Baseline volume (mL)		1.017 (1.001-1.002)	<0.001	1.03 (1.02-1.04)	**<0.001**
Nodule structure	S	1.00 (ref)		1.00 (ref)	
	PS	0.45 (0.20-0.98)	0.04	0.51 (0.11-2.39)	0.39
	PC/C	0.33 (0.88-1.35)	0.12	0.91 (0.13-6.71)	0.93
Nodule function	AFTN	1.00 (ref)			
	Non-AFTN	1.59 (0.58-4.36)	0.38	//	//
1-year reduction		0.03 (0.02-0.06)	<0.001	0.04 (0.02-0.09)	**<0.001**
Regrowth	No	1.00 (ref)		1.00 (ref)	
	Yes	2.00 (1.16-3.19)	0.003	1.68 (0.99-2.87)	**<0.001**
Energy/volume (J/mL)		0.99 (0.99-1.00)	<0.001	0.99 (0.99-1.00)	**0.04**

Multivariate model was performed including parameters assessed in the univariable analysis with a *p*-value of less than the prespecified cut-off of 0.10. AFTN is for autonomously functioning thyroid nodules, S is for solid, C is for cystic, PC is for predominantly cystic, and PS is for predominantly solid.

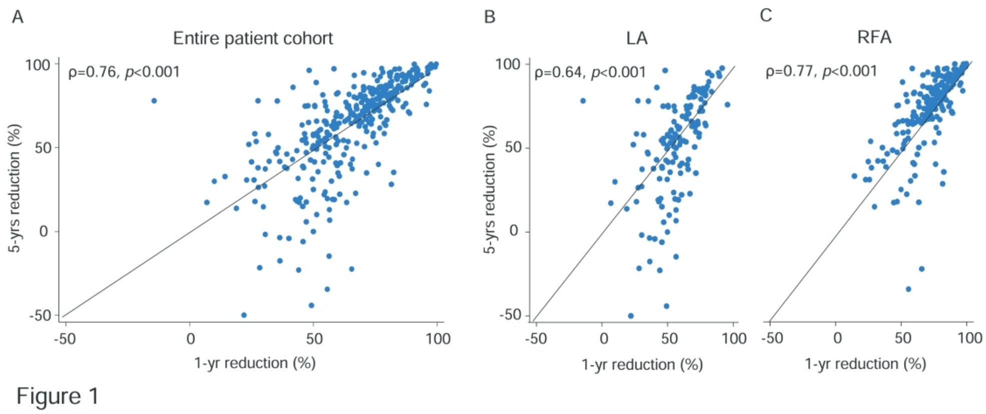


Figure 1

179x75mm (600 x 600 DPI)

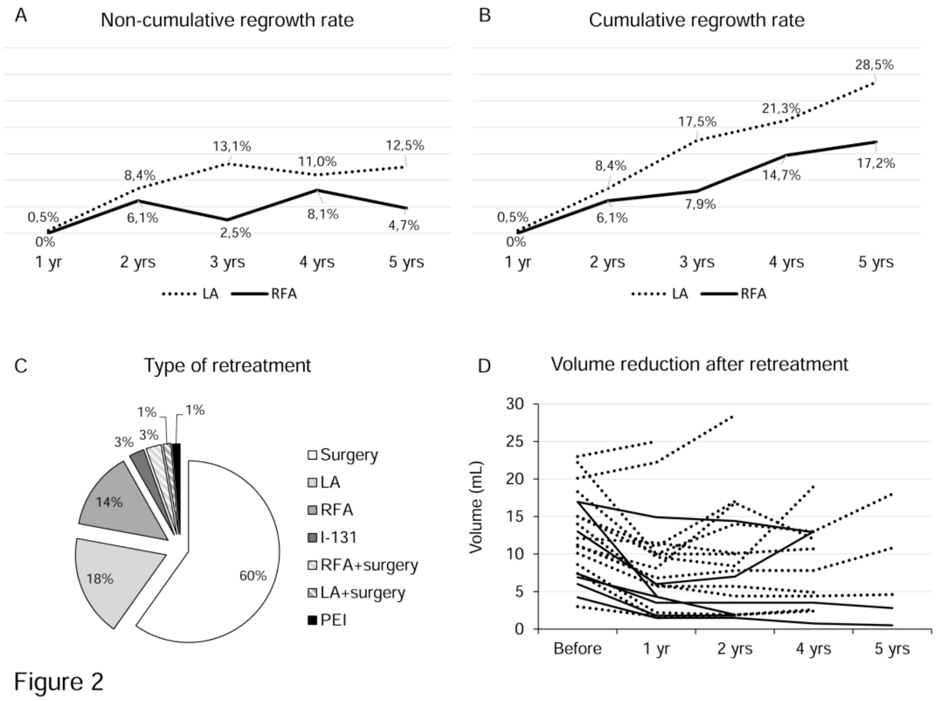


Figure 2

170x123mm (600 x 600 DPI)

A

1-yr VRR (%)	LA (N=190)	RFA (N=216)	*p*-value
<50 (no treatment efficacy)	71 (37.4%)	33 (15.3%)	<0.001
50-60	48 (25.3%)	33 (15.3%)	
60-70	29 (15.3%)	29 (13.4%)	
70-80	32 (16.8%)	48 (22.2%)	
80-90	8 (4.2%)	46 (21.3%)	
≥90	2 (1.1%)	27 (12.5%)	

B

1-yr VRR (%) after PSM	LA (N=76)	RFA (N=76)	*p*-value
<50 (no treatment efficacy)	32 (42.1%)	14 (18.4%)	<0.001
50-60	19 (25.0%)	13 (17.1 %)	
60-70	9 (11.8%)	7 (9.2%)	
70-80	14 (18.4%)	15 (19.7%)	
80-90	2 (2.6%)	18 (23.7%)	
≥90	0 (0.0%)	9 (11.8%)	

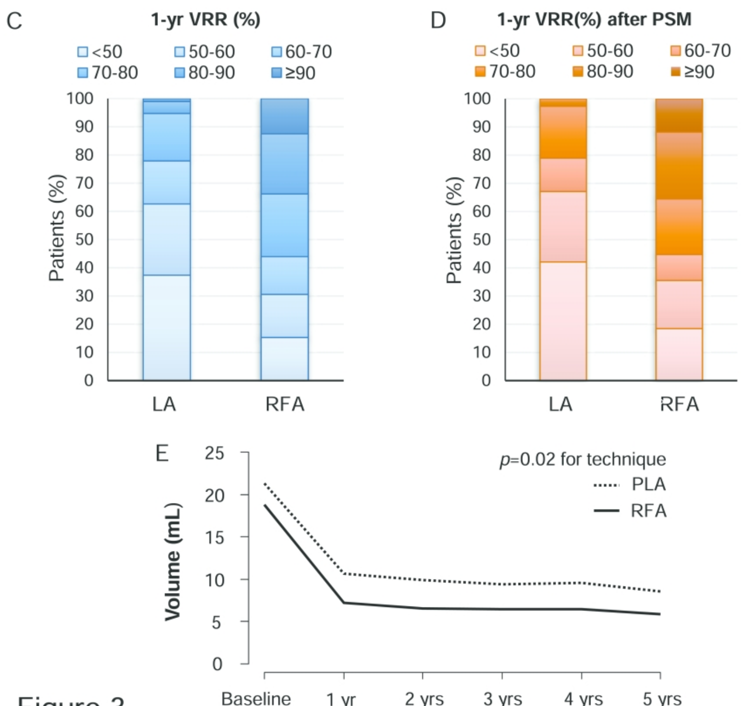
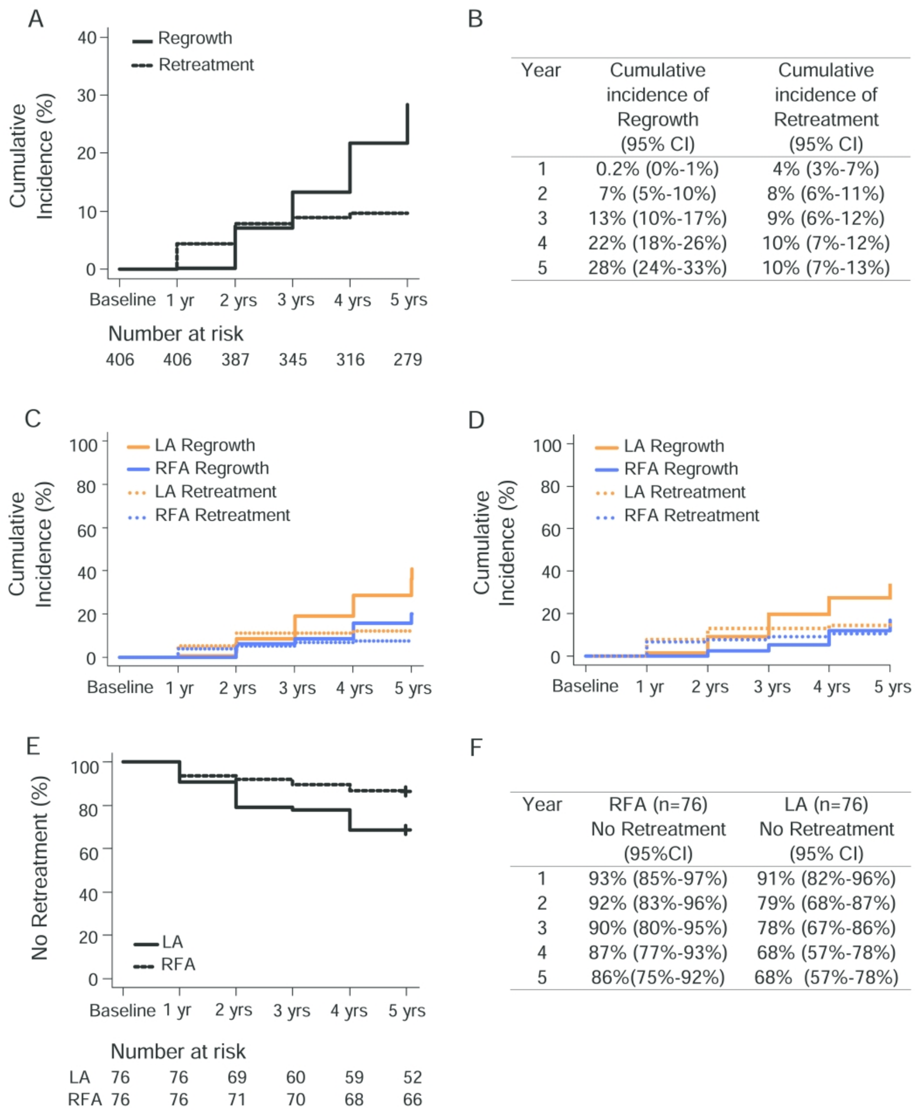


Figure 3

Figure 3

149x223mm (600 x 600 DPI)



Year	Cumulative incidence of Regrowth (95% CI)	Cumulative incidence of Retreatment (95% CI)
1	0.2% (0%-1%)	4% (3%-7%)
2	7% (5%-10%)	8% (6%-11%)
3	13% (10%-17%)	9% (6%-12%)
4	22% (18%-26%)	10% (7%-12%)
5	28% (24%-33%)	10% (7%-13%)

Year	RFA (n=76) No Retreatment (95%CI)	LA (n=76) No Retreatment (95% CI)
1	93% (85%-97%)	91% (82%-96%)
2	92% (83%-96%)	79% (68%-87%)
3	90% (80%-95%)	78% (67%-86%)
4	87% (77%-93%)	68% (57%-78%)
5	86%(75%-92%)	68% (57%-78%)

Figure 4

Figure 4

149x194mm (600 x 600 DPI)

Supplementary Table 1. Centers, techniques, consecutive annual cohorts, and number of patients included and excluded.

Center	Technique	Patient cohorts	End of 5-year follow-up	Patients included (n=406)	Patients excluded (n=71)	
					Not meeting criteria (n=12)	Lost (n=59)
Genova	RFA	2012-2014	2017-2019	19	0	12
Latina	RFA	2014	2019	37	0	1
Lecce	LA	2009-2014	2014-2019	87	0	26
Milano	RFA	2014	2019	17	0	1
Napoli	RFA	2013	2018	24	0	0
Teramo	LA	2009-2014	2014-2019	103	0	2
Torino	RFA	2014	2019	40	5	4
Trieste	RFA	2012-2014	2017-2019	79	7	13

Supplementary Table 2. Characteristics of RFA and LA groups after propensity score matching analysis

	RFA (n=76)	LA (n=76)
Age (range), years	58.5 (33-85)	63.5 (29-78)
Female (%)	57 (75.0%)	55 (72.4%)
Baseline volume (mL)	15.9 (1.2-67.0)	17.5 (2.5-86.0)
Solid nodules (%)	76 (100.0%)	76 (100.0%)
Non-functioning nodules (%)	63 (82.9%)	73 (96.1%)
Energy/volume (J/mL)	1397.9 (175.6-2409.8)	348.1 (61.0-1100.4) *

*p<0.001 with Mann-Whitney Test

Supplementary Table 3. Logistic regression models for technique inefficacy, regrowth, and retreatment

		TECHNIQUE INEFFICACY			
		Univariate logistic regression		Multivariate logistic regression	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age (years)		1.00 (0.98-1.01)	0.49	//	//
Sex	Male	1.00 (ref)			
	Female	1.01 (0.63-1.69)	0.97	//	//
Baseline volume (mL)		1.01 (0.99-1.02)	0.06	1.01 (0.99-1.02)	0.49
Nodule structure	S	1.00 (ref)			
	PS	0.37 (0.18-0.76)	0.01	1.12 (0.39-3.23)	0.84
	PC/C	0.08 (0.01-0.65)	0.02	0.21 (0.03-1.78)	0.15
Nodule function	AFTN	1.00 (ref)			
	Non-AFTN	2.09 (0.77-5.55)	0.14	//	//
Energy/volume (J/mL)		0.99 (0.99-0.99)	<0.001	0.99 (0.99-0.99)	**<0.001**
		REGROWTH			
		Univariate logistic regression		Multivariate logistic regression	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age (years)		0.98 (0.97-0.99)	0.01	0.99 (0.98-1.01)	0.60
Sex	Male	1.00 (ref)		1.00 (ref)	
	Female	1.73 (1.01-2.96)	0.04	1.57 (0.89-2.89)	0.15
Baseline volume (mL)		0.98 (0.97-0.99)	0.04	0.99 (0.98-1.01)	0.28
Nodule structure	S	1.00 (ref)			
	PS	0.66 (0.36-1.23)	0.19	//	//
	PC/C	0.96 (0.49-1.28)	0.92		
Nodule function	AFTN	1.00 (ref)		1.00 (ref)	
	Non-AFTN	3.21 (1.10-9.34)	0.03	3.00 (0.36-25.20)	0.31
1-year reduction (%)		0.71 (0.30-1.68)	0.44	//	//
Energy/volume (J/mL)		0.99 (0.99-1.00)	<0.001	0.99 (0.99-1.00)	**0.001**
		RETREATMENT			
		Univariate logistic regression		Multivariate logistic regression	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age (years)		0.98 (0.96-0.99)	0.01	0.96 (0.93-0.98)	**0.005**
Sex	Male	1.00 (ref)			
	Female	0.99 (0.55-1.79)	0.98	//	//
Baseline volume (mL)		1.03 (1.01-1.04)	<0.001	1.06 (1.04-1.09)	**<0.001**
Nodule structure	S	1.00 (ref)		1.00 (ref)	
	PS	0.41 (0.18-0.95)	0.04	0.34 (0.05-2.20)	0.26
	PC/C	0.30 (0.07-1.30)	0.11	1.18 (0.10-14.00)	0.89
Nodule function	AFTN	1.00 (ref)			
	Non-AFTN	1.66 (0.56-4.87)	0.36	//	//
1-year reduction		0.006 (0.002-0.02)	<0.001	0.005 (0.0008-0.03)	**<0.001**
Regrowth	No	1.00 (ref)		1.00 (ref)	
	Yes	2.42 (1.43-4.10)	0.001	3.54 (1.58-7.57)	**0.002**
Energy/volume (J/mL)		0.99 (0.99-0.99)	<0.001	0.99 (0.99-1.00)	**0.07**

Multivariate model was performed including parameters assessed in the univariable analysis with a *p*-value of less than the prespecified cut-off of 0.10. AFTN is for autonomously functioning thyroid nodules, S is for solid, C is for cystic, PC is for predominantly cystic, and PS is for predominantly solid.

Supplementary Table 4. Odds ratios for non-benign pathology

NON-BENIGN PATHOLOGY			
		Univariate logistic regression	
		OR (95% CI)	*p*-value
Age (years)		1.02 (0.98-1.06)	0.29
Sex	Female	1.00 (ref)	
	Male	5.75 (1.36-24.4)	**0.0177**
Baseline volume (mL)		0.99 (0.97-1.02)	0.80
Nodule structure	S	1.00 (ref)	
	C/PC	0.0011 (0.00-NA)	0.99
	PS	5.31 (0.50-56.40)	
1-year reduction (%)		0.54 (0.08-3.58)	0.521
Regrowth	No	1.00 (ref)	
	Yes	0.49 (0.13-1.79)	0.28
Energy/volume (J/mL)		1.00 (0.99-1.00)	0.36

S is for solid, C is for cystic, PC is for predominantly cystic, and PS is for predominantly solid.