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RESEARCH

Radiofrequency ablation is an effective treatment for Bethesda III thyroid nodules without genetic alterations

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Abstract

Background: Radiofrequency ablation (RFA) is effective in the treatment of thyroid nodules, leading to a 50–90% reduction with respect to baseline. Current guidelines indicate the need for a benign cytology prior to RFA, though, on the other side, this procedure is also successfully used for the treatment of papillary microcarcinomas. No specific indications are available for nodules with an indeterminate cytology (Bethesda III/IV).

Aim: To evaluate the efficacy of RFA in Bethesda III nodules without genetic alterations as verified by means of a custom panel.

Methods: We have treated 33 patients (mean delivered energy 1069 ± 1201 J/mL of basal volume) with Bethesda III cytology, EU-TIRADS 3-4, and negative genetic panel. The mean basal nodular volume was 17.3 ± 10.7 mL.

Results: Considering the whole series, the mean volume reduction rate (VRR) was 36.8 ± 16.5% at 1 month, 59.9 ± 15.5% at 6 months, and 62 ± 15.7% at 1-year follow-up. The sub-analysis done in patients with 1 and 2 years follow-up data available ($n = 20$ and $n = 5$, respectively) confirmed a progressive nodular volume decrease. At all-time points, the rate of reduction was statistically significant ($P < 0.0001$), without significant correlation between the VRR and the basal volume. Neither cytological changes nor complications were observed after the procedure.

Conclusion: RFA is effective in Bethesda III, oncogene-negative nodules, with reduction rates similar to those obtained in confirmed benign lesions. This procedure represents a good alternative to surgery or active surveillance in this particular class of nodules, regardless of their initial volume. A longer follow-up will allow to evaluate further reduction or possible regrowth.

Keywords: Bethesda III; gene mutations; indeterminate; radiofrequency; thyroid nodule

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Introduction

Radiofrequency ablation (RFA) is an effective treatment for thyroid nodules, with a mean volume reduction ranging 50–90% at 1-year follow-up (1). As a general rule, the smaller the treated nodule the higher the volume reduction (78–82% in ≤10–12 mL nodules vs 62–65% in >20–30 mL nodules) (2). Besides the association with the baseline nodular volume, it has been shown that the volume reduction rate (VRR) is significantly associated with the delivered energy (3).

According to the European Thyroid Association guidelines (ETA GLs) for image-guided ablation in benign thyroid nodules, one or two benign cytologic results are needed before the procedure, and the treatment is not recommended for nodules with high-risk US features (EU-TIRADS Class 5) (2). On the other hand, in the ETA GLs for the use of minimally invasive techniques in malignant lesions, RFA has been recommended as therapeutic alternative for the treatment of papillary microcarcinomas and as palliative treatment in advanced and infiltrative thyroid tumors (4). No specific recommendations are available regarding the use of RFA or other mini-invasive procedures in indeterminate nodules (Bethesda III and IV). According to the last Bethesda classification (5), nodules classified as atypia of undetermined significance or follicular lesion of undetermined significance – AUS/FLUS, Bethesda III – harbor a risk of malignancy of 22% (range: 13–30%) and the recommended management is to repeat cytology or to perform a molecular testing, or a diagnostic lobectomy, or an active surveillance. Few papers have been published reporting the efficacy of thermo- or microwave ablation in this class of nodules, but only small (volume range: 1.9–7.9 mL) and genetically uncharacterized nodules have been treated (6, 7, 8). Our group recently demonstrated in a large mono-institutional series that nodules with a Bethesda III cytology, of any EU-TIRADS grade, any size and with a negative genetic evaluation, harbor a 10% risk of malignancy (9).

We thus aimed to evaluate for the first time the efficacy of the RFA procedure in Bethesda III nodules of any size and previously found negative at the genetic evaluation done by a wide custom panel.

Patients and methods

All patients had a complete clinical, hormonal, and B-mode ultrasonography (US) evaluation. Patients harboring at least one nodule with a solid or mixed US pattern with a solid portion >70%, EU-TIRADS 2-4, without US or clinical suspicious features, and with at least one cytology corresponding to Bethesda III classification were considered eligible to enter the study. They were informed about the possibility to be followed with an active surveillance (asymptomatic nodules <10 mL), to be submitted to lobectomy/total thyroidectomy (nodules >10 mL uni-or bilateral, or symptomatic), or to be treated by RFA. Those patients selecting RFA were consecutively enrolled and submitted to the genetic analysis. Only patients with normal thyroid function and normal serum calcitonin concentrations were enrolled.

The enrolment started in June 2020 and ended in June 2022. This prospective study was conducted in accordance with the Declaration of Helsinki and received the Istituto Auxologico Italinao IRCCS Ethical Committee's approval (#2020_05_19_02). Written consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used.

RFA technical data

The median power expressed in watts, energy obtained by kilocalorie (calculated by the generator) × 4184 and expressed in joules, and the energy deposition duration were reported. Both total energy applied and energy delivered per milliliter of basal volume in J/mL were assessed.

Ultrasound parameters

The ultrasound scans and color Doppler scans were obtained by two commercially available ultrasound systems (Esaote Eight-Plus and Esaote MyLab Twice) equipped with a linear transducer operating at 7.5–12 MHz for the morphological study and 4.7 MHz for the ultrasound evaluation with color Doppler imaging. The nodular volume was estimated by using the ellipsoid formula (i.e., 0.52 × anteroposterior × latero-lateral × longitudinal diameters). At 1 month, 6 months, 1 year, and 2 years an ultrasound evaluation was performed, and theVRR, expressed as percentage, was calculated by applying the following formula: ((initial volume − final volume)/initial volume) ×100. Nodules were classified by the EU-TIRADS scoring system (10).

Genetic analysis

The genetic analysis of fine-needle aspirate (FNA) samples was done as previously described (11). Briefly, genomic DNA and total RNA were extracted from FNA samples using AllPrep DNA/RNA Micro Kit (Qiagen). To test the follicular cell content of the FNA sample, PAX8 expression on cDNA was evaluated by semiquantitative RT-PCR. According to the sensitivity of the PTC-MA, a thyroid cell content of 10% was chosen to select samples adequate for further molecular analyses. DNA and cDNA were analyzed using the custom PTC-MA (acronym for Papillary Thyroid Cancer-Masa Array) assay, based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and previously set up for the simultaneous identification of 13 known hotspot mutations (BRAFV600E; AKT1E17K; EIF1AX c.338-1G>C; NRASQ61R and NRASQ61K; HRASG13C, HRASQ61K, and

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HRASQ61R; KRASG12V and KRASG13C; TERT c.-124C>T and TERT c.-146C>T; PIK3CAE542K and six recurrent fusion genes typical of PTC: RET/PTC1 (RET/CCDC6), RET/PTC2 (RET/PRKAR1A), and RET/PTC3 (RET/NCOA4); TRK (NTRK1/TPM3), TRK-T1 (NTRK-T1/TPR), and TRK-T3 (NTRK1/TFG); and PAX8_8/PPARγ, PAX8_9/PPARγ, and PAX8_10/PPARγ fusions (11). In cases negative for any mutations/fusions analyzed by PTC-MA assay, BRAFK601E mutation was investigated by direct sequencing of exon 15 (12), and AKAP9/BRAF and LMO7/BRAF fusions were analyzed on cDNA, as previously reported (13, 14).

Study protocol

The RFA treatment sessions were carried out between October 2020 and October 2022. The clinical evaluation, thyroid hormones and calcitonin assessment, and US were performed 1–4 weeks before RFA treatments and these were used as the basal data. An indirect laryngoscopy was performed before RFA treatment and after treatment only in cases symptomatic for a possible nerve injury. The patients were treated with 2% lidocaine for local anesthesia at the puncture site, and RFA was done as previously reported (15). Briefly, under ultrasound guidance, we performed RFA using the trans-isthmic approach and the "moving-shot" technique (16), paying attention to the preservation of important surrounding structures, such as laryngeal nerves. We used an RFA system (RFA needle tube system, RF Medical, Seoul, Korea, or VIVA STARmed®, Seoul, Korea) and an internally cooled 18-gauge electrode with an active 10-mm tip. Each nodule underwent a single treatment session and was followed up over time. Immediately after the procedure, all nodules were evaluated using contrast enhanced ultrasound to evaluate the boundaries of the induced necrosis. RFA was performed on an outpatient basis. Patients were discharged 2 h after RFA treatment. Follow-up after RFA was scheduled at 1, 6, 12, and 24 months. Thyroid hormone measurements and US were performed at each follow-up visit. Measurement of thyroid nodule volume before and after RFA was performed always by the same operator, who was blind to the previous volume measurements. Laboratory examinations comprised measurements of serum TSH (normal range: 0.35–3.6 mIU/L), free T4 (normal range: 8–15 pg/mL), and calcitonin (normal range: 0–10 pg/mL), antithyroid antibodies titers (<115 IU/mL), and platelet count and blood coagulation tests (including prothrombin time and activated partial thromboplastin time). In five patients (#1, 8, 11, 14, 18), selected among patients accepting to be re-biopsed for a scientific purpose and having an adequate follow-up, a cytology was repeated 1-year after RFA. FNA was done in the undertreated peripheral portion of the nodule.

Statistical analysis

The statistical analysis was carried out by Chi-square, *t*-test, and correlation coefficient analysis, as appropriate. Data are reported as mean ± s.d. The significance was set at $P < 0.05$.

Results

Baseline features

According to the study design, 33 nodules were included and treated, 21 at the Istituto Auxologico Italiano IRCCS, and 12 at the Mauriziano Hospital. All nodules had a Bethesda III cytology and were negative at the oncogene panel analysis. Twenty-one patients had a uninodular goiter, and 12 patients had a multinodular goiter either mono or bilateral.

Among the baseline clinical features, there was a predominance of female sex ($n = 24$), the mean age was 52.7 ± 14.7 years (range: 18–77), and the nodules were classified as EU-TIRADS 3 in 28 cases and EU-TIRADS 4 in 6 cases. The mean basal nodule volume was 17.3 ± 10.7 mL (median: 13.8 mL, IQR: 18.5, range: 1.6–40.1 mL), the mean total energy delivered was 47.2 ± 10.7 watts (median: 50 ± 20 watts) or 19.3 ± 9.8 kJ (median: 16.7 ± 16.5 kJ), with a mean 1069 ± 1201 J/mL of basal nodular volume (median: 1098 ± 1393). The mean duration of treatment was 9 min:00 s ± 3 min:48 s (median: 9 min:1 s, range: 1 min:14 s–16 min:37 s) (Table 1).

Efficacy

The VRR at 1 month after RFA was available for the 33 included patients, 6 months VRR for 30 cases, 1-year VRR for 20 patients, and 2-year VRR for five patients (Table 2). Considering all cases, at the 1-month evaluation, the mean nodular volume was 11.1 ± 7.9 mL (median: 8.3, IQR: 10.9), with a mean VRR of 36.8 ± 16.5% (median: 38.6, IQR: 21.2). At the 6-month evaluation, the mean nodular volume was 7.3 ± 6 mL (median: 4.9, IQR: 7.4), with a mean VRR of 59.9 ± 15.5% (median: 60.5, IQR: 17.7), and at 1 year after treatment the mean nodular volume was 7.9 ± 5.8 mL (median: 6.2, IQR: 8.9), with a mean VRR of 62 ± 15.7% (median: 60.7, IQR: 16.6). A reduction <50% (21.3%, 41.4%, 44.1%, and 47.8%) was found only in 4 patients (#6, 11, 15, and 21) who had a thyroid nodule volume ranging 21.5–34.5 mL. Finally, the five patients evaluated at 2 years of follow-up (#1, 2, 3, 8, and 14) had a mean nodular volume of 8.8 ± 2.9 mL (median: 7.7, IQR: 4.1), with a mean VRR of 66.9 ± 4% (median: 64.8, IQR: 3.9). The VRRs were statistically significant ($P < 0.0001$) at each follow-up time with respect to basal volume (Table 2, Fig. 1).

When considering separately the data obtained in the 20 patients with a 1-year follow-up, the nodule size progressively decreased up to the last control, with the mean basal volume 19.5 ± 11.2, the 1-month volume 12.6 ± 6.3, the 6-month volume 9 ± 8.1, and the 1-year volume 7.9 ± 5.8 mL, with a VRR of 38.1 ± 13.9%, 55.7 ± 15, and 62 ± 15.7% at 1 month, 6 months, and 1 year of

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Table 1 Basal characteristics of the patients included in the study.

ID*	Sex	Age	EU-TIRADS	BV (mL)	Watts delivered	kJ delivered	Duration of energy deposition (min)	J/mL of BV
1	M	46	4	16.4	35	13.9	10.32	841
2	F	45	3	32.7	40	20.1	10.02	613
3	M	55	4	33.2	40	18.4	9.16	554
4	M	49	3	34.2	60	36.7	16.11	1071
5	F	64	3	16.1	35	7.9	5.44	490
6	F	63	3	21.5	30	10.7	8.19	496
7	M	18	3	26.9	55	19.9	8.03	740
8	M	51	3	28.8	50	13.6	6.08	470
9	F	45	3	9.2	50	10.8	5.05	1173
10	F	63	3	12.1	30	10.4	7.59	857
11	F	59	3	34.5	50	12.9	6.29	374.5
12	F	19	4	1.6	30	5.6	3.54	3525
13	F	32	3	7.3	40	11.7	9.42	1600
14	F	50	3	20.8	35	16.7	12.12	804
15	M	24	3	32.4	50	35.9	16.37	1106
16	F	45	3	8.8	50	9.7	4.09	1098
17	M	64	3	24	35	12.7	9.10	530
18	F	40	3	8.4	30	8.0	6.19	955
19	F	42	3	13.5	35	12.4	11.48	917
20	F	42	3	40.1	50	33.4	15.58	833
21	F	46	3	27.1	45	24.0	11.42	900
22	M	63	3	14.8	65	31.5	13.03	2126
23	F	68	4	5.6	60	18.9	7.23	3385
24	F	64	3	7.7	60	28.2	9.04	3657
25	F	56	3	18	65	39.5	13.29	2197
26	F	59	4	13.6	60	31.4	11.14	2304
27	F	67	3	4.8	50	10	3.51	5555
28	F	63	4	13.8	55	30.3	12.21	2198
29	F	71	3	7.1	50	13.8	6.25	1945
30	F	60	3	11.3	50	22.3	9.06	1970
31	F	70	3	6.1	60	19.8	7.01	3244
33	M	77	3	6	50	12.2	6.19	2022
33	F	58	3	13	55	33.1	13.23	2549
Mean ± S.D.	–	52.7 ± 14.7	–	17.3 ± 10.7	47.1 ± 10.9	19.3 ± 9.8	9.00 ± 3.48	1069 ± 1201
Median ± IQR	–	56 ± 18	–	13.8 ± 18.5	50 ± 20	16.7 ± 16.5	9.06 ± 5.24	1098 ± 1393

*Patients 1–22 were treated at the Istituto Auxologico Italiano, Milan and patients 23–34 were treated at the Mauriziano Hospital, Turin, Italy.
F: female, M: male; BV, basal volume.

follow-up. The mean nodule volume of the 5 patients with a 2-year follow-up decreased from 28.8 mL up to 7.7 mL with a maximum VRR at 2 years of 64.8% (Fig. 2).

No significant correlation was found between the VRR and the basal volume of the nodules (R = 0.33, P = 0.126). On the other hand, we found a significant correlation between the reduction rate and the energy delivered (R = –0.52, P = 0.012).

No major complications occurred in the patients treated.

Cytological assessment after RFA

In the five patients who were resubmitted to cytology 6–12 months after RFA, the cytological pattern was similar to that found before RFA, and the diagnosis of Bethesda III nodule was confirmed (Fig. 3).

Discussion

We report for the first time that a single session of RFA represents a good alternative to surgery or active surveillance in patients with indeterminate and oncogene negative nodules of any volume. Since up to the 15% of thyroid nodules harbors a Bethesda III cytology, our findings represent a significant and cost-effective innovation in clinical practice, allowing to avoid surgery and its potential complications in a high number of patients.

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Table 2 One month, 6 months, 1-year, and 2-year volume reduction rate (VRR) with respect to the basal volume (BV).

		1 month		6 months		1 year		2 years	
ID*	BV (mL)	Volume (mL)	VRR (%)	Volume (mL)	VRR (%)	Volume (mL)	VRR (%)	Volume (mL)	VRR (%)
1	16.4	5.2	−68.3	4.8	−70.7	4.6	−71.9	5.2	−68.3
2	32.7	15.8	−51.7	10.3	−68.5	9.9	−69.7	11.5	−64.8
3	33.2	22.6	−31.9	15	−54.8	15.3	−53.9	12.1	−63.5
4	34.2	21	−38.6	14.6	−57.3	13.3	−61.1	–	–
5	16.1	9.3	−42.3	6.5	−59.6	6.4	−60.2	–	–
6	21.5	15.9	−26.0	13	−39.5	12.6	−41.4	–	–
7	26.9	23.2	−13.8	–	–	–	–	–	–
8	28.8	24.5	−14.9	16.5	−42.7	12.8	−55.5	7.7	−73.3
9	9.2	6.1	−33.7	3.9	−57.6	–	–	–	–
10	12.1	6.4	−47.1	4.8	−60.3	4.2	−65.3	–	–
11	34.5	30.8	−10.7	20.2	−41.4	–	–	–	–
12	1.6	1	−37.5	0.8	−50.0	0.7	−56.2	–	–
13	7.3	5.6	−23.3	3.6	−50.7	–	–	–	–
14	20.8	11.7	−43.7	9.8	−52.9	8.6	−58.6	7.4	−64.4
15	32.4	24.2	−26.0	19.8	−38.9	18.1	−44.1	–	–
16	8.8	6.7	−23.9	–	–	–	–	–	–
17	24	16.2	−32.5	–	–	–	–	–	–
18	8.4	4.1	−51.2	3.3	−60.7	3.8	−54.8	–	–
19	13.5	8.3	−38.5	7.2	−46.7	6	−55.5	–	–
20	40.1	21.1	−47.4	10.5	−73.8	9.3	−76.8	–	–
21	27.1	16.4	−22.2	18.5	−12.7	16.6	−21.3	–	–
22	14.8	8.4	−43.2	5.9	−60.1	1.8	−87.8	–	–
23	5.6	2.6	−55.9	1.0	−83.7	0.9	−84.7	–	–
24	7.7	5.3	−31.2	2.2	−71.4	1.3	−83.1	--	–
25	18	9.5	−47.2	6.0	−66.7	4.3	−76.1	–	–
26	13.6	8.3	−39.0	5.0	−63.2	4.0	−70.6	–	–
27	4.8	1.6	−66.7	0.7	−85.4	–	–	–	–
28	13.8	5.2	−62.3	3.0	−78.3	–	–	–	–
29	7.1	7.6	+7	1.3	−81.7	–	–	–	–
30	11.3	8.8	−22.1	3.8	−66.4	–	–	–	–
31	6.1	3.1	−49.2	2.0	−67.2	–	–	–	–
33	6	3.6	−40	2.1	−65.0	–	–	–	–
33	13	7.8	−40	4.0	−69.2	–	–	–	–
Mean ± SD.	17.3 ± 10.7	11.1 ± 7.9	−36.8 ± 16.5	7.3 ± 6	−59.9 ± 15.5	7.9 ± 5.8	−62 ± 15.7	8.8 ± 2.9	−66.9 ± 4
Median ± IQR	13.8 ± 18.5	8.3 ± 10.9	−38.6 ± 21.2	4.9 ± 7.4	−60.5 ± 17.7	6.2 ± 8.7	−60.7 ± 16.6	7.7 ± 4.1	−64.8 ± 3.9

*Patients 1–22 were treated at the Istituto Auxologico Italiano, Milan, and patients 23–34 were treated at the Mauriziano Hospital, Turin, Italy.

The VRR was statistically significant with respect to the initial volume even at 1 month and reached a mean reduction of 60–62% at 6 and 12 months of follow-up. The observed volume reduction was similar to that observed for benign nodules, which ranges 50–90%, with higher shrinkages in nodules <10 mL (1). The relevant efficacy in small nodules has been also reported in a recent paper from Taiwan showing an 87.4% VRR at 1 year in non-genetically characterized Bethesda III nodules with a 7.92 mL basal volume (8). The original findings of the present study, in which the 75% of the lesions treated were >10 mL (mean basal volume: 17.3 ± 10.7 mL, range: 1.6–40.1 mL), reside in the use of a low-cost custom panel and in the demonstration that RFA is effective in indeterminate nodules of any size, without correlation between the VRR and the basal volume. This latter finding reflects the high expertise of the participating centers which led to the appropriate treatment of all nodules, independently from the initial volume. In the same context, as observed for benign nodules (17), we found a significant correlation between the delivered energy and the rate of reduction, confirming in our series of indeterminate nodules that this technical parameter has a pivotal role, and efforts should be done to treat also large nodules with an appropriate energy.

In the subgroups of patients evaluated at 1 and 2 years, a progressive volume decrease was observed, though a longer follow-up is needed since regrowth from marginal undertreated tissue is frequently reported 2–3 years after RFA (18). Nevertheless, data at the long follow-up of 2 years, although obtained in a limited number of

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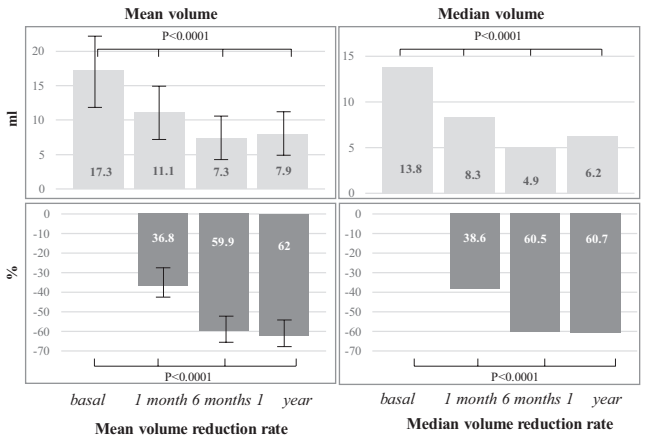

Figure 1

Mean and median volume (upper panels) and volume reduction rate (lower panels) after radiofrequency ablation and considering the whole series at baseline, 1 month, 6 months, and 1 year of follow-up.

cases, seem to indicate that the regrowth of this kind of nodules is null or extremely limited.

According to previous findings in benign nodules (19), we demonstrated in some representative cases that RFA does not induce cytologic changes, arguing against a possible transformation of the tissue adjacent to the treated area and further supporting the safety of this procedure even in indeterminate nodules.

One of the main strengths of the present study was that similar results were obtained by two different tertiary-level institutions which worked according to the same protocol and using the same devices, indicating the reliability of the data obtained with this standard RFA procedure.

We can envisage some potential drawbacks of this study. The first relates to the use of a comprehensive, but not full, coverage of the oncogenes possibly involved in thyroid carcinogenesis. Nevertheless, the panel used, despite a relatively low cost (around 300 euros), covers the vast majority of the mutations associated to thyroid cancers including those responsible for the more aggressive phenotypes and has shown a high specificity and sensitivity (9). Our confidence on the extremely low or null risk of malignancy in our treated nodules is to strengthen by the observation that for the 131 Bethesda III nodules with negative genetics presently on active surveillance in our center, a mean increase of less than 1.5 mm has been documented at ultrasound after a minimum follow-up of 10 months (data beyond the scope of this study and not shown). The second pitfall resides in the lack of a very long-term follow-up, which could reveal unexpected effects of RFA on nodules of a malignant but undiagnosed origin. We believe that this possibility is virtually null considering that no detrimental effects have been reported in malignant nodules treated with RFA and that the serial ultrasonographic follow-up usually allows to detect the possible development of suspicious features. Finally, only a minority of patients were re-biopsed after RFA. Nevertheless, the literature does not report indications for a cytology after the procedure, since RFA is not expected to give rise to malignant transformation, as demonstrated by Ha *et al.* in a series of benign nodules (19).

In conclusion, we show that one RFA session is effective in the volume reduction of Bethesda III nodules whose very low risk of malignancy has been established on

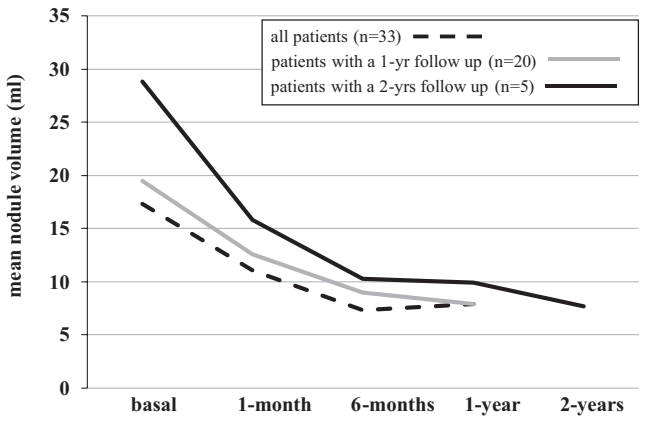

Figure 2

Volume reduction trend from baseline in the whole series, and in the groups of patients with 1-year and 2-year follow-up evaluations after radiofrequency ablation.

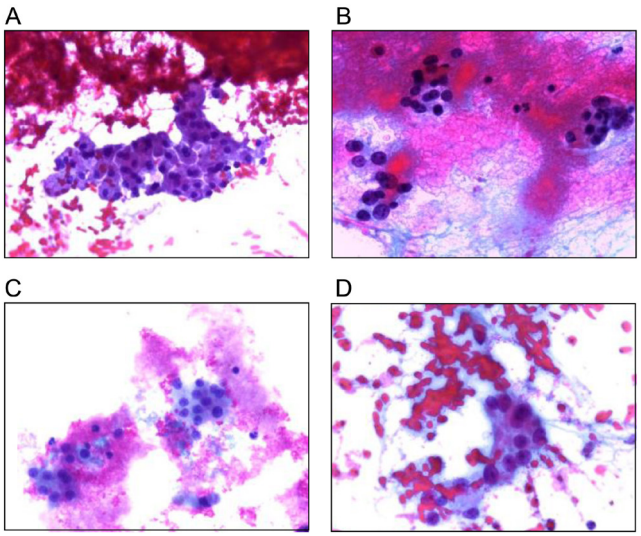

Figure 3

Two exemplificative cases showing the cytological samples of patients 11 and 18, before and 1-year after the radiofrequency ablation procedure. (A) patient 11 pre-RFA: microfollicular pattern with oxyphilic cells (conventional cytology 20×); (B) patient 11 post-RFA: microfollicular pattern with oxyphilic cells (liquid-based cytology 40×); (C) patient 18 pre-RFA: microfollicular pattern with oxyphilic cells (conventional cytology 40×); (D) patient 18 post-RFA: microfollicular pattern with oxyphilic cells (conventional cytology 40×).

the bases of the lack of the most common oncogene variations. The procedure is applicable for indeterminate nodules of any size, treated with an appropriate energy per volume and the volume reduction is maintained at 1–2 years after treatment, but a longer follow-up is needed to identify a further reduction or a possible progressive regrowth.

Finally, future studies will establish if the present finding could be applied also to patients treated with different protocols/procedures and can be extended to Bethesda IV nodules.

Declaration of interest

L Fugazzola and L Persani are on the Editorial Board of the ***European Thyroid Journal***. They were not involved in the peer-review process of this article. All other authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported here.

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