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ORIGINAL ARTICLE

Combined clinical and ultrasound follow-up assists in malignancy detection in Galectin-3 negative Thy-3 thyroid nodules

Salvatore Sciacchitano[1,2] · Luca Lavra[2] · Alessandra Ulivieri[2] · Fiorenza Magi[2] · Tommaso Porcelli[1] · Stefano Amendola[1] · Gian Paolo De Francesco[3] · Carlo Bellotti[4] · Maria Concetta Trovato[5] · Leila B. Salehi[2,6] · Armando Bartolazzi[7,8]

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Abstract The use of galectin-3 ThyroTest in the preoperative evaluation of cytologically indeterminate (Thy-3) thyroid nodules has been largely validated by retrospective and prospective multicentre studies. Here we report the results of galectin-3 ThyroTest routinely applied in the management of Thy-3 nodules in combination with clinical and ultrasonography (US) examination, in which galectin-3 positive nodules were directly referred to surgery whereas galectin-3 negative lesions were considered for clinical and US long-term follow-up. A cohort of 331 patients, bearing 340 thyroid Thy-3 nodules, was enrolled and subjected to galectin-3 expression analysis. A total of 256 galectin-3 negative nodules were directed to periodical clinical and US examination, while 84 galectin-3 positive cases were referred to surgery. Excluding 63 dropout patients plus 15 patients that were operated because of clinical reasons the remaining 176 galectin-3 negative nodules were followed with clinical and US examination for an average period of 31 months. During the follow-up, the volume of galectin-3 negative nodules was unchanged in 85 cases (48 %), reduced in 47 (27 %), and increased in 44 (25 %). Based on combined clinical features and US follow-up results, a total of 36 out of 191 galectin-3 negative nodules (19 %) were referred to surgery, with a final histological finding of 28 benign lesions, three follicular tumor of uncertain malignant potential (FT-UMP), and five malignant lesions, corresponding to a 7 % false negative rate. In the group of 84 galectin-3 positive nodules, we detected 65 thyroid cancers with a prevalence of 77 %, 12 FT-UMPs, and 7 false positive lesions, corresponding to a 4 % false positive rate. A total of 150 patients were not operated and are still under clinical and US monitoring while surgery was performed in 118 patients with a final 70 thyroid cancers diagnosed, corresponding to a 59 % prevalence of malignancy detected at surgery and to a 26 % prevalence of malignancy among the entire Thy-3 nodule population. Galectin-3 ThyroTest is an easy and cheap diagnostic procedure that integrates conventional fine-needle-aspiration cytology, reduces the number of unnecessary thyroidectomies and increases the

✉ Salvatore Sciacchitano
ssciacchitano@hotmail.it;
salvatore.sciacchitano@fondazioneniccolocusano.it

[1] Department of Clinical and Molecular Medicine, Sapienza University, Policlinico Umberto I, Viale Regina Elena n. 324, 00161 Rome, Italy

[2] Laboratorio di Ricerca Biomedica, Fondazione Università Niccolò Cusano per la Ricerca Medico Scientifica, Via Don Carlo Gnocchi 3, 00166 Rome, Italy

[3] Department of Oncological Science, Breast Unit, St Andrea University Hospital, Via di Grottarossa, 1035/39, 00189 Rome, Italy

[4] Operative Unit Surgery of Thyroid and Parathyroid, Sapienza University of Rome, S. Andrea Hospital, Via di Grottarossa, 1035/39, 00189 Rome, Italy

[5] Department of Clinical and Experimental Medicine, University of Messina, Policlinico Universitario "G. Martino", via Consolare Valeria, Gazzi, 98125 Messina, Italy

[6] Department of Biopathology and Diagnostic Imaging, Tor Vergata University, Via Montpellier, 1, 00133 Rome, Italy

[7] Laboratory of Surgical and Experimental Pathology, St Andrea University Hospital, Via di Grottarossa, 1035/39, 00189 Rome, Italy

[8] Department of Oncology-Pathology, Cancer Center Karolinska Universitetssjukhuset Solna, 17176 Stockholm, Sweden

rate of malignancy at surgery. Clinical and US follow-up of galectin-3 negative lesions allows to further reduce false negative cases.

Keywords Thyroid cancer diagnosis · FNA cytology · FNA indeterminate · Galectin-3

Introduction

The *gray zone* of indeterminate thyroid nodules is one of the major limits of fine-needle aspiration (FNA) cytology. Currently, surgery is suggested in all these lesions to verify their nature at final histology. However, malignancy is finally found in less than 30 % of these thyroid nodules [1, 2]. Therefore, more than 70 % of thyroid patients with indeterminate nodules are currently addressed to surgery to remove a benign lesion. Many attempts have been made to ameliorate the accuracy of conventional cytology in this group of nodules, using new blood assay [3] as well as imaging techniques [4] and new emerging molecular diagnostic tests [5–7]. Galectin-3 is a multifunctional protein involved in regulation of apoptosis as well as in tumor invasion and metastasis [8–11] and a potential role as therapeutic target for this molecule has been suggested [12–16]. In TAD-2 cultures of normal thyroid follicular cells galectin-3 overexpression is sufficient to induce a malignant phenotype [17]. Its expression is also necessary for the maintenance of transformed phenotype in NPA papillary thyroid carcinoma cells [18]. Galectin-3 is specifically expressed in the cytoplasm of transformed thyroid follicular cells and for this reason its potential utility in the diagnosis of thyroid tumors was initially suggested by Xu [19]. Its use as an ancillary method for improving the diagnostic accuracy of thyroid FNA cytology was validated in large retrospective and prospective studies [20, 21]. However, despite the strong biological rationale of galectin-3 expression in transformed thyroid follicular cells [8] and its diagnostic performance in the preoperative characterization of thyroid nodules, its clinical use is still restricted to few specialized thyroid institutions.

The present study was conducted to assess the clinical utility of a combined clinical and ultrasonographic approach to increase accuracy of galectin-3 test-method, when routinely applied in the preoperative evaluation of cytologically indeterminate thyroid nodules, especially when the test gives a negative result.

Materials and methods

Study group

All patients with thyroid nodules, which were classified as indeterminate at conventional cytology, were initially evaluated by ultrasonography (US), using a high-resolution ultrasound apparatus (MyLab^{TM}25, MyLab^{TM}70 XVG and MyLab^{TM}Alpha, Esaote, Genova, Italy), equipped with a 12.5 MHz linear probe (LA523). The volume of the lesion was measured using the formula of the rotation ellipsoid ($V_{ellipsoid} = \pi/6 \times D_{length} \times D_{width} \times D_{depth}$). Suspicious nodules were subjected to routine FNA sampling. Only patients, harboring thyroid nodules, cytologically classified as Thy-3, according to the British Thyroid Association (BTA) [22], including follicular thyroid proliferations with and without atypia as well as Hurthle cell follicular proliferations, were enrolled for the study. Written informed consent was obtained from each enrolled patient. The study was approved by the local Ethics Committee (Prot. CE n. 938/2013 and Prot. CE n. 8391/2013).

Thyroid FNA cytology and galectin-3 ThyroTest

FNA cytology under US guidance was performed as previously described [23]. One FNA sample was obtained from each thyroid nodule (maximum diameter ranging from 0.4 to 5.6 cm). Cell block preparation was performed using FNA-derived cytological material as previously described [24]. Briefly, thyroid cells obtained by FNA were collected into a 15 mL Falcon tube filled with 8–10 mL of physiological saline solution or PBS, centrifuged gently at 700–1000 RPM for 10 min and the supernatant was decanted off. The pellet was re-suspend in 3–4 drops of plasma. Few drops (3–4) of thromboplastin and few drops (3–4) of calcium chloride were added, and then mixed gently for a faster fibrin clot reaction. Then 5–10 mL of buffered formalin was added and samples were incubated for 12–24 h. to allow them to fix. After spinning down gently, the clot were placed in a labeled tissue cassette and processed like a small tissue biopsy for cellblock preparation.

Slides obtained from sections of cellblocks were stained in haematoxilin/eosin for the routine histo-cytological evaluation. Diagnosis was rendered according to BTA guidelines [22]. No distinction was made concerning the two subcategories of Thy-3, namely the Thy-3f (with follicular pattern) and Thy-3a (with atypia). In all cellblocks of cytological samples classified as Thy-3, a consecutive 4 μm tissue section was prepared and considered for galectin-3 expression analysis, using a purified mAb to human galectin-3 HRP (horseradish peroxidase)-conjugated (Mabtech, Nacka, Sweden), at a concentration range of 5-10 mg/mL. Antigen retrieval was obtained by microwave treatment at 750 W in 0.01 M citrate buffer, at pH 6 for 3 cycles of 3–5 min each. Two pathologists, in blind, rendered cytological diagnosis and immunophenotypical characterization for each thyroid nodule. Galectin-3 immunostaining was considered positive when follicular thyroid cells showed galectin-3 accumulation in the

cytoplasm, with or without nuclear staining. In the absence of cytoplasmic staining for galectin-3, samples were considered negatives. Thyroid foamy macrophages that constitutively express galectin-3 were used as internal positive controls.

Thyroid surgery and histological evaluation

When surgery was indicated a total thyroidectomy was performed. In case of small nodules (<2 cm in diameter), a minimally invasive video-assisted total thyroidectomy (MIVAT) technique was applied. Resected thyroid glands, containing the nodules object of the study, were histologically evaluated by two independent pathologists in blind, according to the BTA guidelines [22]. In selected cases (galectin-3 false positive or false negative results), galectin-3 expression analysis was performed also post-operatively on the corresponding histological samples for comparative evaluation.

US follow-up of galectin-3 negative nodules

In order to minimize undiagnosed malignancy, patients with galectin-3 negative nodules (170 patients with 176 nodules) were followed with periodical clinical examination and US evaluation (every 6 months), as currently suggested in case of FNA-proven benign nodules. Every thyroid nodule was measured in its three diameters to calculate its volume by applying the rotation ellipsoid formula. US evaluations were performed using the same US apparatus and by the same operator (SS) in order to reduce inter-operator variations of thyroid nodule measurements. Changes in nodule volumes were recorded and expressed as percentage of monthly variation, using an arbitrary cut-off point at 1 % per month. Approximately 70 % of patients, with galectin-3 negative nodules, were subjected to levothyroxine (LT4) treatment at suppressive dosage.

Statistical analysis

The performances of the different diagnostic tests have been calculated as previously reported [20, 21]. Patients with follicular tumors with undefined malignant potential (FT-UMP) were excluded from statistical analysis because they are considered indeterminate also at final histology by definition.

For each reported test-method sensitivity, specificity, PPV, NPV, FPR, FNR, FDR, LH+, and LH−, Cancer risk rate in positive test and Cancer risk rate in negative test were calculated, as well as three measures of diagnostic test accuracy, namely accuracy, F1 score and DOR. The following formulas were used for calculations: sensitivity or true positive rate (TPR) = TP/(TP + FN), negative predictive value (NPV) = TN/(TN + FN), cancer risk in negatives test = FN/(FN + TN), false negative rate (FNR) = FN/(FN + TP) negative likelihood ratio (LR-) = (FN/(TP + FN))/(TN/(FP + TN)), specificity or true negative rate (TNR) = TN/(TN + FP), cancer risk in positives test or positive predictive value (PPV) = TP/(TP + FP), false positive rate (FPR) = FP/(FP + TN) positive likelihood ratio (LR +)=(TP/(TP + FN))/(FP/(FP + TN)) accuracy = (TP + TN)/(P + N), F1 score = 2TP/(2TP + FP + FN), and diagnostic odds ratio (DOR) = (TP/FN)/(FP + TN).

All statistical measures of the diagnostic performances have been obtained by assuming that all nodules unchanged or reduced in their volume during the follow-up are benign, and excluding 23 nodules that showed a significant increase in their volume (>1 % per month) and that have not been operated yet.

The χ^2 test has been used to analyse the correlation between galectin-3 ThyroTest results and the different clinical patients characteristic and between monthly volume variation of galectin-3 negative nodules and LT4 treatment.

Results

Galectin-3 ThyroTest in the surgical selection of thyroid nodules

Galectin-3 expression analysis was performed in a total of 340 thyroid nodules, classified as Thy-3 at cytology, obtained from 331 thyroid patients (Fig. 1). The test was positive in 84 cases (25 %), all referred to surgery, while 256 thyroid nodules (75 %) were found to be galectin-3 negative and considered for follow-up. As expected we didn't observe any statistically significant difference in terms of nodule characteristics, including nodule volume, or patient characteristics between galectin-3 positive and negative lesions (Table 1).

In the group of 84 galectin-3 positive nodules, the final histology confirmed the presence of thyroid cancer in 65 cases (77 %), whereas 12 thyroid lesions were classified as FT-UMP, for which surgery was anyway recommended. The remaining seven cases were diagnosed as benign thyroid proliferations and included six follicular adenomas (FA) and one Hashimoto's thyroiditis (Table 2). Presence of scattered galectin-3 positive cells in FA, mostly located at sub-capsular level, is not sufficient *per se* to make a diagnosis of follicular thyroid carcinoma (FTC), because no capsular nor vascular invasion was detected at histology. Surgery was also performed in 15 galectin-3 negative nodules because of clinical reasons (compressive

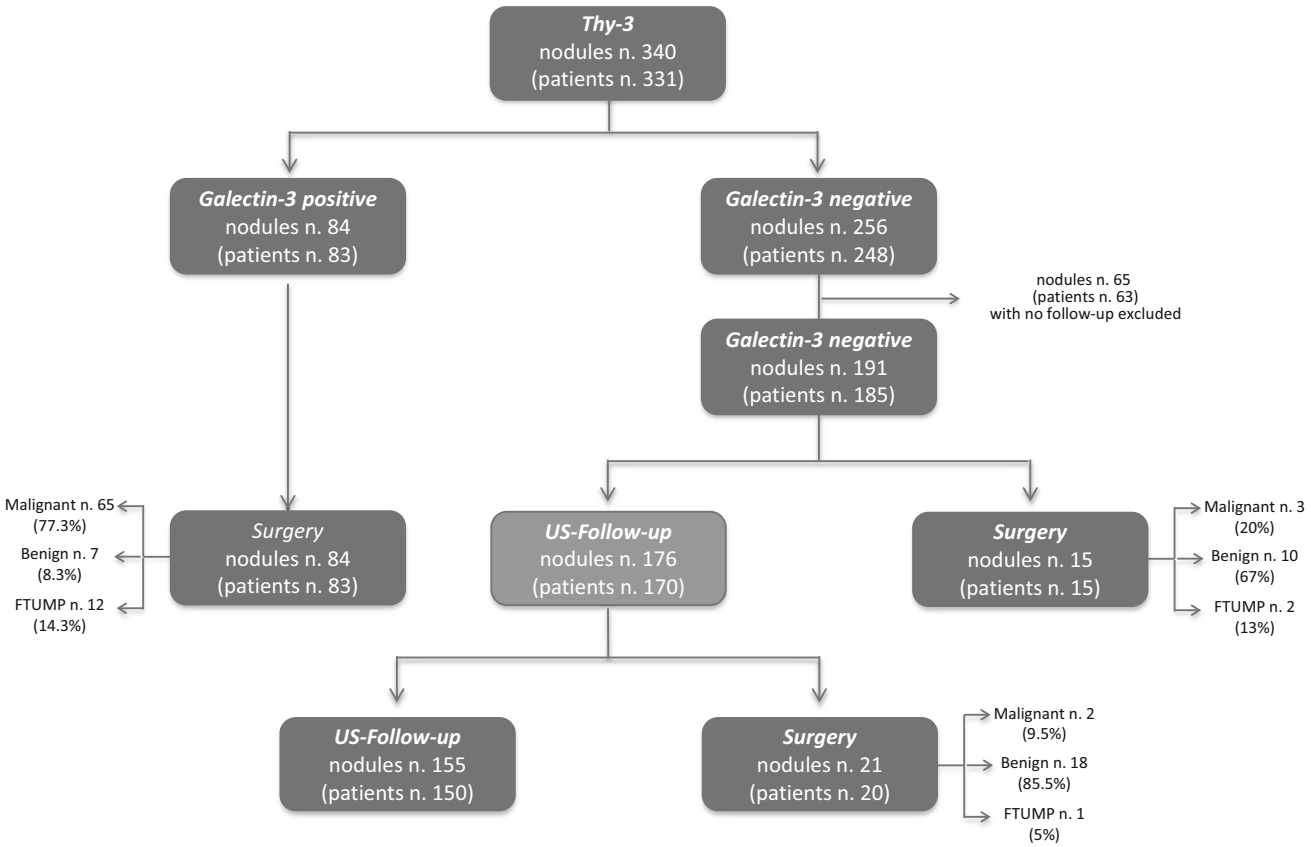

Fig. 1 Flow schematic representation of Thy-3 thyroid nodules managed in the study

symptoms and/or hyperthyroidism). In these cases, two follicular variant papillary thyroid carcinomas (FVPTCs), one medullary thyroid carcinoma (MTC), and two FT-UMPs were discovered (Fig. 1; Table 2).

The combined use of galectin-3 ThyroTest and US follow-up improves the preoperative selection of Thy-3 nodules candidate for surgery

To further increase recognition of malignancy, we followed patients bearing galectin-3 negative nodules by performing periodical clinical and US evaluations for an average period of 31 months (min. 6, max. 131, and median 29 months) (Fig. 2). A group of 63 galectin-3 negative patients dropped out from the follow-up and were excluded from the study. Among the remaining 170 patients, harboring 176 nodules, nodule volume was reduced (<1 % per month) in 47 cases (26.7 %), remained stable in 85 cases (48.3 %) and was increased (>1 % per month) in 44 cases (25.0 %) (Fig. 3). LT4 treatment was administered approximately in the same percentage of patients in each group, without any substantial difference in term of nodule volume reduction between treated and non-treated patients. In particular, LT4 treatment was given in 69 % of patients with decreased, in 69 % with unchanged and in 67 % with increased nodule volume. In the latter group, surgery was performed in 21 cases and two thyroid malignancies (FTC oncocytic variant and FV-PTC) and one FT-UMP were detected (Figs. 1, 3; Table 2). Considering the 84 galectin-3 positive nodules, plus the 15 galectin-3 negative nodules, selected on the basis of clinical reasons, and the 21 nodules showing increase in their volume during US follow-up, we performed a total of 118 thyroidectomies to remove 70 thyroid cancers. The rate of malignancy detected at final histology was 59 %, much higher than that (24.4 %) reported in a most representative Italian study [2], in which all Thy-3 cases underwent to surgery. galectin-3 ThyroTest, combined with clinical and US evaluation, therefore, reduces the number of thyroidectomies performed to remove benign lesions and, at the same time, increases the rate of thyroid cancer detected at surgery. A total of 128 patients, bearing 132 nodules, were not subjected to surgery and they are still under observation, with the examined nodule stable (85 cases) or reduced (47 cases) in its volume.

The use of galectin-3 based ThyroTest combined with clinical and ultrasound may, therefore, assists in malignancy detection, especially in galectin-3 negative Thy-3 thyroid nodules.

Table 1 Characteristic of patients and nodules included in the study

	Total		Galectin-3 negative		Galectin-3 positive	
	n	%	*n*	%	*n*	%
Patients characteristics						
Total number	331		248		83	
Female	261	79	194	78	67	81
Male	70	21	54	22	16	19
Age (years)						
Mean	53		54		52	
Median	53		54		51	
Range	20–88		20–84		20–88	
<50	130	40	94	38	36	43
50–60	107	32	82	33	25	30
>60	94	28	72	29	22	27
Single Thy-3 nodule	324		242		82	
Multiple Thy-3 nodules	7		6		1	
Two Thy-3 nodules	6		5		1	
Four Thy-3 nodules	1		1		0	
Nodules characteristics						
Total number	340		256		84	
Nodule size (maximum diameter, cm)						
Mean	1.8		1.8		1.9	
Median	1.5		1.5		1.7	
Range	0.4–5.6		0.5–5.6		0.4–4.8	

Diagnostic pitfalls of galectin-3 ThyroTest

Among 256 galectin-3 negative nodules, 36 of which were surgically treated, we detected a total of five false negative results, which represents 7 % of the 70 cancers detected. The clinical and cytomorphological features of these thyroid lesions, including the reasons why they were removed, are reported in Table 3. In three out of four of these cases, the final histology revealed the presence of one minimally invasive, oncocytic type FTC, and three FV-PTC. Interestingly, in two of them galectin-3 immunostaining, performed on histological samples, revealed the presence of galectin-3 at the periphery of the lesions, whereas the central region of the tumor was largely galectin-3 negative, in another case galectin-3 positive cancer cells were located inside a largely galectin-3 negative hyperplastic nodule. In all these instances, FNA samplings of these nodules were not fully representative of the lesion. In another false negative case, a multifocal FV-PTC showed a consistent expression of galectin-3 on histological samples, suggesting the occurrence of sub-optimal FNA sampling or technical problems in cellblock processing and/or immunostaining. The remaining false negative case consisted in a MTC, not recognized at cytology, with negative galectin-3 expression at immunohistochemistry, an event already reported to occur in 50 % of these tumors [20]. The concomitant presence of elevated calcitonin serum levels enabled us to promptly refer this patient to surgery, independently by the negative galectin-3 expression analysis. Two of the five galectin-3 false negative carcinomas were detected among the 21 thyroid nodules increased in their volume during US follow-up.

A total of seven galectin-3 false positive results were detected in 118 thyroidectomies (Table 2). In six of them final histology was FA, in which scattered and heterogeneous galectin-3 positive thyroid cells were also detected at immunohistochemistry, mostly at sub-capsular level. The possibility that these FA may represent early precursors of well-differentiated thyroid carcinomas is intriguing, but deserves further investigations. It should be considered, however, that surgical option for such cases does not represent *per se* a real overtreatment. The remaining galectin-3 false positive lesion was a nodular follicular hyperplasia, occurring in Hashimoto's thyroiditis. The possibility of focal galectin-3 expression during Hashimoto's thyroiditis has been previously reported, leading to possible surgical overtreatment [20].

Cost saving of combined use of galectin-3-based ThyroTest with clinical and US follow-up

Another major aim of this study was to assess the cost saving of galectin-3 ThyroTest, in the preoperative

Table 2 Clinical and histological features of Galectin-3 ThyroTest false negative and false positive cases

#	Age/sex	Nodule site	Nodule diameter (cm)	Reasons for surgery	Final histology	Galectin-3 IHC
Galectin-3 false negative cases						
1	58 F	Right	3.6	Increased nodule volume (42 % of initial total volume, equal to 1.4 % per month)	MIFTC oncocytic	Galectin-3 + (variable at the periphery)
2	47 F	Left	1.4	Elevated serum CT	MTC	Galectin-3 -
3	72 F	Right	0.9	Compressive symptoms	FVPTC	Galectin-3 + (variable at the periphery)
4	62 F	Right	1.0	Additional controlateral nodule Thy-4	FVPTC multifocal	Galectin-3+
5	48 F	Left	2.0	Increased nodule volume (480 % of initial total volume, equal to 14 % per month)	FVPTC	Galectin-3+
Galectin-3 false positive cases						
1	54 M	Right	4·8	Galectin-3 + ThyroTest	FA	Galectin-3 + (scattered at the periphery)
2	42 M	Right	4·0	Galectin-3 + ThyroTest	FA	Galectin-3 + (scattered at the periphery)
3	60 F	Left	1·0	Galectin-3 + ThyroTest	FA	Galectin-3 + (scattered at the periphery)
4	60 M	Left	1·0	Galectin-3 + ThyroTest	FA	Galectin-3 + (scattered at the periphery)
5	66 F	Left	4·0	Galectin-3 + ThyroTest	FA oncocytic	Galectin-3 + (scattered at the periphery)
6	50 M	Right	2·7	Galectin-3 + ThyroTest	FA oncocytic	Galectin-3 + (scattered at the periphery)
7	51 F	Right	1·0	Galectin-3 + ThyroTest	HT	Galectin-3 + (focal)

FA follicular adenoma, *HT* Hashimoto's thyroiditis, *MIFTC* minimally invasive follicular thyroid carcinoma, *FVPTC* follicular variant papillary thyroid carcinoma, *MTC* medullary thyroid carcinoma, *CT* calcitonin

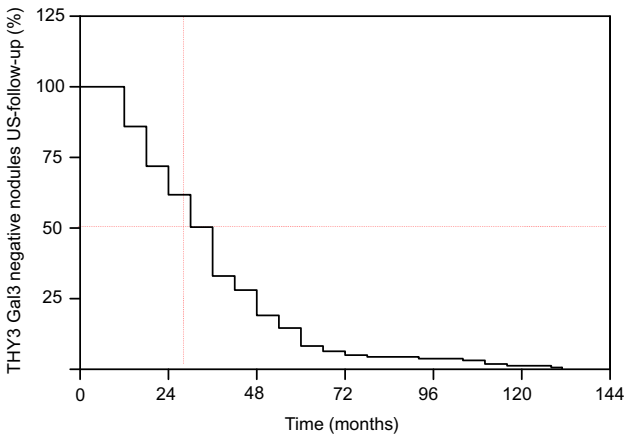

Fig. 2 Graph of follow-up of patients enrolled in the study. A medium follow-up of 31 months has been obtained

diagnosis of thyroid nodules when used in combination with clinical and US follow-up. Our diagnostic approach enabled us to accurately select, among Thy-3 lesions, those more likely to be classified as malignant at the final histology. As a consequence unnecessary thyroid surgeries were dramatically reduced. Considering that a single galectin-3 ThyroTest costs 112 USD, equal to 100 EUR, we calculated a total expense of 34,000 EUR for all ThyroTest performed in the cohort of Thy-3 nodules object of this study.

In the absence of galectin-3 ThyroTest all 331 patients would have been referred to surgery. Using a combination of galectin-3 ThyroTest, clinical, and US follow-up, a total of 148 operations (45 %) have been avoided. Considering the reimbursement of 3300 EUR for each thyroidectomy (excluding any complication), by our National Health System, we may estimate a total saving of 488,400 EUR. An extrapolation of these data into a wider National context confers an impressive economical impact to the application of galectin-3 ThyroTest.

Discussion

It is well known that clinical data, such as age, sex, or nodule dimension as well as other features are of little help in predicting malignancy in patients bearing Thy-3 nodules [25, 26]. Recently, an algorithm has been proposed for the clinical management of Thy-3 nodules [7]. In this algorithm, the most effective test-method in ruling-out malignancy, namely the Veracyte, *Afirma*® gene expression classifier, was proposed as a screening test-method in all Thy-3 nodules, while the use of the other new molecular test-method, namely the Asuragen miR*Inform*™ multiple gene mutation panel, should be restricted to confirm malignancy in Thy-4 nodules, commonly referred to surgery, for better planning the extent of surgery (i.e., lobectomy *vs* total thyroidectomy). Galectin-3 ThyroTest performed well both as an efficient rule-out and rule-in test-method, with a rather good diagnostic accuracy. Based on its diagnostic odds ratio, accuracy, and cost, galectin-3

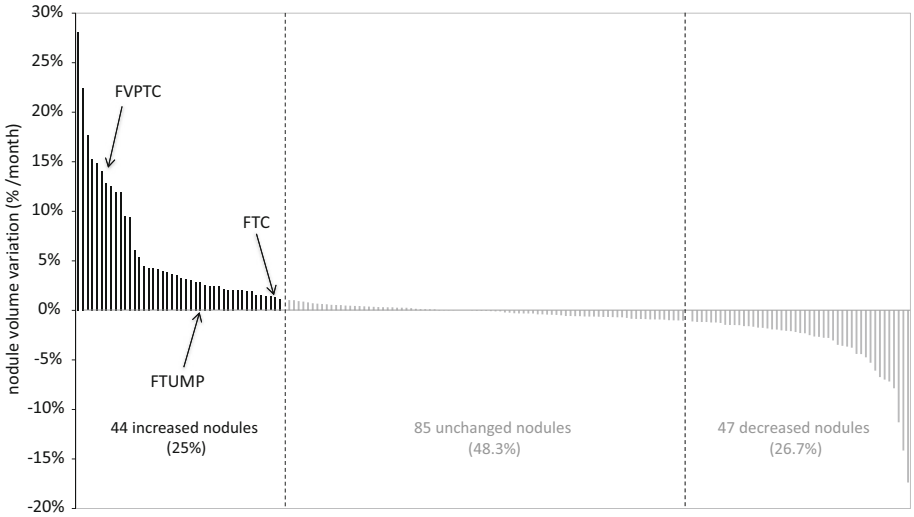

Fig. 3 Analysis of nodule volume variation observed during the US follow-up of galectin-3 ThyroTest negative patients, examined at 6 months intervals. The percentages of monthly nodule volume variations are reported for the 176 nodules in follow-up. Arrows indicate three nodules that were surgically treated and resulted a follicular variant of papillary thyroid carcinoma (FVPTC), a follicular thyroid carcinoma (FTC) and a follicular tumor of uncertain malignant potential (FT-UMP)

Table 3 Diagnostic performances of galectin-3 ThyroTest

	Galectin-3 ThyroTest (Bartolazzi et al. [21])
Sensitivity (%)	78
Specificity (%)	93
Positive predictive value (%)	82
Negative predictive value (%)	91
False positive rate (%)	7
False negative rate (%)	22
False discovery rate (%)	18
Diagnostic accuracy (%)	88
F1 score (%)	80
Diagnostic odds ratio	44
Positive likelihood ratio	11
Negative likelihood ratio	0.24
Cancer risk rate (%) in positive test	82
Cancer risk rate (%) in negative test	7

The performance of the galectin-3 ThyroTest alone was calculated using data reported in the multicentric and prospective study previously published (Bartolazzi et al. [21])

ThyroTest represents the best candidate test-method to be used in a large-scale basis. Moreover, galectin-3 ThyroTest uses conventional FNA cytological substrates, is very easy to be performed in different clinical settings and does not require to be centralized in high specialized laboratories. For all these reasons, galectin-3 ThyroTest appears to be a suitable screening test-method for the preoperative characterization of Thy-3 nodules. However, the major drawback of this test-method was represented by the occurrence of few, but noticeable, false negative results, that affected its sensitivity (78 %), its cancer risk rate in negative test (9 %), its false negative rate (22 %), and reduced its overall accuracy (85 %) and its diagnostic odds ratio (44) [21]. It appears that the major advantage of this test-method relies in its low cost, but there is still place for improvement in its diagnostic performances. In the present study we applied, in a real clinical setting, the galectin-3 ThyroTest to evaluate its clinical validity in the management of Thy-3 nodules and to improve its accuracy, especially in the case of a galectin-3 negative results. For this reason, we planned a strict clinical and US follow-up of galectin-3 negative thyroid nodules. We did not find any correlation between age, nodule size, and positivity at the galectin-3 ThyroTest or malignancy at final histology, confirming the little utility of such parameters in predicting malignancy. We based the therapeutic decision on a combined use of galectin-3 ThyroTest results, clinical and US follow-up. As previously suggested, the use of Ultrasounds in the evaluation of thyroid nodules can be advocated as a possible alternative to clinical measurement and to other radiologic measurement such as CT, RX or PET even for the assessment of the response to treatment of solid tumors, i.e., the RECIST criteria [27]. Although it has been considered significant a volume change of at least 49 % of the initial measure [28], we decided to evaluate the changes in nodule volume by calculating the rate of increase in term of percentage of variation per month. Using this approach, a larger number of nodules showing significant volume variations (>1 % per month) during the follow-up were identified, including two galectin-3 false negative cases.

The diagnostic efficacy of galectin-3 ThyroTest in the surgical selection of thyroid nodules was previously evaluated in both retrospective and prospective studies [20, 21]. In the multicenter prospective study, the cumulative cancer risk in test negative cases was 7 % (Table 3) [21]. It should be noted that in that study all patients were surgically

treated and all nodules histologically verified. In the present study, two galectin-3 false negative carcinomas were detected among 21 galectin-3 negative thyroid nodules referred to surgery because of a significant volume increase. We might expect to find two more additional cancers among the remaining 23 nodules, increased but not operated yet. Assuming that no other malignancy would be found among 132 unchanged or decreased nodules, we would expect a total of seven false negative results, with an estimated cancer risk in negative cases of 4 %. This value is still lower than that of 6 % reported for benign lesions (Thy-2) for which no surgery is required but only US follow-up is advised [6]. Nodules that reduce their volume either spontaneously or after suppressive treatment with LT4 are more likely to be benign [29]. On the other hand, a decrease in thyroid nodule size *per se* does not necessarily indicate benign nature of the lesion, as it was even observed in 13–15 % of thyroid cancers treated with LT4 [28]. Successful TSH suppression therapy, however, assumes a functional TSH receptor (TSHR) in thyroid cells a feature associated with benign nodules or eventually more differentiated/less aggressive tumors. In galectin-3 negative Thy-3 nodules that underwent US follow-up, LT4 treatment was performed in a similar percentage of cases in the three groups (increased, unchanged or decreased nodule volume). Shrinkage of the nodule was obtained spontaneously in 26 % of the nodules. These results, therefore, do not indicate LT4 treatment as a possible adjunctive test to verify the biological nature of Thy-3 nodules.

In conclusion, the combined use of galectin-3 ThyroTest with clinical and US follow-up of negative nodules allowed us to further increase the diagnostic accuracy, with a very low additional cost of one thyroid US exam every 6 months. Such approach allowed us to avoid many unnecessary thyroid surgeries as well as to reduce the social costs of thyroid surgery and of possible surgical complications. Considering that the occurrence of Thy-3 nodules at conventional cytology has been reported in approximately 10-40 % of FNA specimens [29], the cost saving offered by the proposed diagnostic approach would result in a great benefit for Thyroid Centers that examine thousands of patients per year. Combination of galectin-3 ThyroTest with clinical and US follow-up, represents the most cost-effective strategy for the management of Thy-3 nodules and should, therefore, be introduced in the routine diagnostic algorithm of these nodules.

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Compliance with ethical standards

Conflict of interest The authors declared no conflict of interest and do not benefit financially from the galectin-3 ThyroTest.

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