Quantitative dual isotope 123iodine/99mTc-MIBI scintigraphy: A new approach to rule out malignancy in thyroid nodules

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Quantitative dual isotope ^{123}iodine/^{99m}Tc-MIBI scintigraphy: a new approach to rule out malignancy in thyroid nodules
Scintigraphie au ^{99m}Tc-MIBI combinée à l'iode 123 : une nouvelle approche pour l'exclusion de malignité des nodules thyroïdiens

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Abstract

Background: The aim of this study was to evaluate the role of dual isotope ^{123}Iodine/^{99m}Tc-MIBI thyroid scintigraphy (IMS) in discriminating between malignant and benign suspect nodules using quantitative analysis methods.

Methods: 35 consecutive patients with thyroid nodules of indeterminate or non-diagnostic cytology and cold on ^{123}Iodine scintigraphy (10 Bethesda I, 24 Bethesda III-IV, 1 in which cytology was impossible) underwent IMS between 2017 and 2019 with uptake quantification at two time points ahead of thyroidectomy: early and late. Images were analyzed by two blinded physicians

Results: 12 nodules were malignant and 23 benign on histopathology. Mean uptake values were lower in benign than in malignant nodules at both time points: early, 8.7±4.1 versus 12.9±3.5 (p=0.005); and late, 5.3 ± 2.7 versus 7.7±1.1 (p=0.008). Inter-observer reproducibility was excellent. The intraclass correlation coefficient was 0.86 in benign and 0.92 in malignant lesions on the early readings and 0.94 and 0.85 respectively on the late readings. The optimal cut-off to exclude malignancy on late reading was 5.9, with sensitivity, specificity, positive predictive value, negative predictive value and accuracy of respectively, 100%, 65.2%, 60%, 100% and 77.1%.

Conclusion: Despite some study limitations, quantitative analysis of ^{99m}Tc-MIBI thyroid scintigraphy had a good reproducibility, which could help to rule out malignancy in non-diagnostic or indeterminate thyroid nodules and thereby reducing the number of patients undergoing unnecessary surgery when late uptake is below 5.9.

Résumé

Contexte : L'objectif de cette étude est d'évaluer l'apport de la Scintigraphie au 99mTc-MIBI combinée à l'Iode 123 (SMI) avec quantification de fixation, dans la discrimination bénin-malin des nodules thyroïdiens (NT) suspects.

Patients et méthodes : 35 patients consécutifs, porteurs d'un NT posant un problème diagnostique de malignité (10 NT Bethesda I, 24 NT Bethesda III-IV, 1 NT d'étude cytologique impossible) ont effectué une SMI avec mesure de la captation du traceur à deux temps d'acquisition précoce (TP) et tardif (TT) avant thyroïdectomie entre 2017 et 2019. L'analyse des images de SMI a été réalisée en aveugle par deux praticiens.

Résultats : 12 nodules étaient malins et 23 bénins en analyse histologique. La moyenne (écart-type) de la mesure de captation du ^{99m}Tc-MIBI était plus élevée dans les cancers au TP (12,9 ± 3,5 *vs.* 8,7 ± 4,1 ; p=0,005) comme au TT (7,7 ± 1,1 *vs.* 5,3 ± 2,7 ; p=0,008). La reproductibilité inter-observateurs était parfaite. Le coefficient de corrélation intra-classe était de 0,86 (lésions bénignes) et 0,92 (lésions malignes) pour le TP, et de 0,94 (lésions bénignes) et 0,85 (lésions malignes) pour le TT. Le seuil optimal de mesure de captation au TT pour l'exclusion de malignité a été fixé à 5,9. La sensibilité, spécificité, valeur prédictive positive, valeur prédictive négative, et précision de ce seuil étaient respectivement de 100%, 65,2%, 60%, 100% et 77,1% pour différencier un NT bénin et malin.

Conclusion : Malgré certaines limites, l'analyse quantitative de la scintigraphie thyroïdienne au ^{99m}Tc-MIBI est reproductible et pourrait contribuer à exclure la malignité des nodules thyroïdiens suspects, réduisant ainsi le nombre de thyroïdectomies inutiles lorsque la mesure de captation au TT est inférieure à 5,9.

Keywords: thyroid nodule, indeterminate or non-diagnostic cytology, ^{99m}Tc-MIBI scintigraphy, malignancy, surgery

Mots clés : nodule thyroïdien, cytologies indéterminées ou non diagnostiquées, scintigraphie au ^{99m}Tc-MIBI, malignité, chirurgie

Background

Thyroid nodules are a common clinical problem. Epidemiologic studies have shown that the prevalence of palpable thyroid nodules is approximately 5% in women and 1% in men living in iodine-sufficient parts of the world [1].

Both European and American guidelines suggest fine-needle aspiration cytology (FNAC) in combination with ultrasonography (US) for the evaluation of malignancy in thyroid nodules [2–4]. Published data suggest that FNAC has a sensitivity ranging from 65% to 98%, a specificity from 72% to 100%, and an overall accuracy of 75% to 90%. Nevertheless, like any other test, FNAC has its limitations and diagnostic traps [5]. In 2–16% of cases, cytology is non-diagnostic and is classified Bethesda category I, i.e., with insufficient material for diagnosis. Moreover, in 5–20% of cases, it is not possible to discriminate between benign and malignant nodules because of an undetermined cytology, including atypia of undetermined significance/follicular lesion of undetermined significance, i.e., Bethesda category III, or because of follicular neoplasm/suspicious for follicular neoplasm, i.e., Bethesda category IV. The risk of malignancy varies from 5% to 10% for non-diagnostic cytology and from 6% to 40% for indeterminate cytology based on the 2017 revision of the Bethesda System for Reporting Thyroid Cytopathology [6]. About 60–95% of patients who underwent thyroidectomy because of non-diagnostic cytology or indeterminate cytology turned out to have a benign lesion at histological evaluation [7,8]. Hence, the use of a non-invasive approach is necessary.

In the last two decades, other methods such as molecular testing, nuclear medicine scans, either alone or in combination (i.e. ^{131}Iodine or ^{123}Iodine or Technetium-99m-methoxyisobutylisonitrile [^{99m}Tc-MIBI] scans combined with either ^{201}Thallium or ^{99m}Tc-methoxyisobutylisonitrile or ^{99m}Tc-tetrofosmin), have been used for that purpose.

^{99m}Tc-MIBI is a lipophilic cation that enters the cytoplasm via thermodynamic driving forces and penetrates the mitochondria using the electrical gradient generated by a high negative inner transmembrane mitochondrial potential. The mechanism of cellular ^{99m}Tc-MIBI accumulation has been reported to depend on the size of a tumor, cellular proliferation, the blood flow within it, and the richness of mitochondria in the tumor cells [9,10].

^{99m}Tc-MIBI has been increasingly used to investigate thyroid nodules in order to differentiate between benign and malignant nodules. However, to assess 99mTc-MIBI retention within the nodules, a majority of authors chose a visual analysis method [11–13]. Two recent meta-analyses with more than 20 studies and including nearly 2000 patients drew similar statements [12,13]. They concluded that ^{99m}Tc-MIBI scintigraphy has good sensitivity (85.1 to 87%) but inconstancy and often poor specificity (45.7 to 78%) for predicting the histological malignancy of thyroid nodules. However, as reported in some prospective studies [14,15], adding a quantitative analysis to assess the tracer kinetic by calculating the retention or washout index may increase diagnostic accuracy.

In many published studies, ^{99m}Tc-MIBI scintigraphy was combined with ^{99m}Tc-pertechnetate, whereas ^{123}Iodine is more sensitive and allows better selection of cold thyroid nodules in comparison with 99mTc-pertechnetate [16].

The aim of this study was to evaluate the diagnostic performance of dual isotope ^{123}Iodine/^{99m}Tc-MIBI scintigraphy using quantitative analysis in discriminating malignant from benign nodules with indeterminate or non-diagnostic cytology, while verifying the inter-observer reproducibility of the technique.

Patients and methods

a) Study design

A retrospective study was conducted at Lille University Hospital, which recruited patients who had undergone exploration of a thyroid nodule that presented diagnostic difficulty. In cases of difficult diagnosis, patients were offered to undergo a dual isotope ^{123}Iodine/^{99m}Tc-MIBI scintigraphy. All patients gave their written informed consent before this procedure. Such cases were discussed in dedicated multidisciplinary meetings. The best treatment option was chosen by the medical and surgical team based on the available guidelines [17] including age, nodule size and progression and the patient's final decision.

Only patients who had been operated were analyzed in this study; they were divided into two groups, benign or malignant thyroid nodules, according to the results of the final pathological analysis after surgery, to assess the performance of dual isotope ^{123}Iodine/^{99m}Tc-MIBI thyroid scintigraphy in discriminating malignant from benign nodules using quantitative analysis.

This study followed the tenants of the Declaration of Helsinki and was approved by the Ethics Committee (N°: DEC19-549) of the Lille University Hospital.

b) Patients

From May 2017 to December 2019, 71 patients were referred from the Endocrinology and Metabolism Department to the Nuclear Medicine Department for thyroid nodule diagnosis with dual isotope ^{123}Iodine/^{99m}Tc-MIBI scintigraphy (Figure 1).

b.1) Inclusion and exclusion criteria

Inclusion criteria

A. TSH levels: 0.4 - 4 mU/L.

B. Nodule > 10 mm in the largest diameter for a multinodular goiter, predominantly solid on US.

C. A cytological diagnosis of class I, III or IV according to the Bethesda classification system; or FNAC impossible due to the position of the nodule.

Exclusion criteria

A. Indifferent and hot nodules at ^{123}I evaluation on dual isotope scintigraphy.

B. Non-operated patients.

c) Study outcomes

All patients underwent physical examination, biological thyroid assays (TSH, FT4, FT3), thyroid US, FNAC (except one patient with an inaccessible nodule) and dual isotope ^{123}Iodine/^{99m}Tc-MIBI scintigraphy.

c.2.1) Ultrasonography

Ultrasound imaging of the thyroid gland and neck region was performed with a high frequency linear ultrasound transducer (ATOSHIBA/Aplio XG™ SSA790A). Thyroid nodules were assessed according to the European Thyroid Imaging Reporting and Data System (EU-TIRADS) classification (EU-TIRADS 1: normal, EU-TIRADS 2: benign, EU-TIRADS 3: low risk of malignancy, EU-TIRADS 4: intermediate risk of malignancy, EU-TIRADS 5: high risk of malignancy) [18].

c.2.2) Fine-needle aspiration cytology

Fine-needle aspiration cytology was indicated according to the criteria recommended by the European and French Societies of Endocrinology [2,4]. FNAC was performed with US guidance. Cytological results were classified according to the Bethesda system [6].

c.2.3) Dual isotope scintigraphy

Dual isotope scintigraphy was carried out in the same day. The total exam time was two and half hours with a radiation dose of about 7.7 millisieverts (mSv) mSv, equivalent to the dose received during a non-injected computed tomography of the abdomen.

Thyroid scintigraphy was carried out using a gamma camera equipped with a low-energy pinhole collimator. Radioiodine (5.5 MBq of ^{123}I) was injected first, and ^{99m}Tc-MIBI (555 MBq)

was injected 90 minutes later. Dual isotope static scans in the anterior projection of the neck were performed 20 minutes (early scan) and 60 minutes (late scan) after ^{99m}Tc-MIBI injection. The acquisition time was set at 600 seconds with a 15% window centered on the 140keV peak and a second 15% window centered on the 163keV peak. The focal length (distance between the collimator and the patient's neck) was constant (6 cm) and checked each time.

In ^{123}Iodine scintigraphy, thyroid nodules were assessed as being either 1) hyperfunctioning, i.e., hot (uptake in the nodule is greater than uptake in normal thyroid tissue); 2) indifferent, i.e., warm (uptake in the nodule is equal to uptake in normal thyroid tissue); or 3) hypofunctioning, i.e., cold (uptake in the nodule is less than uptake in normal thyroid tissue).

^{99m}Tc-MIBI scintigraphy was always interpreted comparatively with ^{123}Iodine scintigraphy to exclude indifferent and hot nodules. Scintigraphies were analyzed by two nuclear medicine physicians (AB & LD) who were blinded for clinical data and results of the cytological analysis.

Quantitative analysis

^{123}Iodine and ^{99m}Tc-MIBI images (early and late) were displayed on the same screen for better precision in drawing the region of interest (ROI). A quantitative analysis was also performed by drawing the ROI around the nodule and then mirroring the ROI outside the thyroid to subtract the background activity (figure 1 in supplementary data). ROIs were created on early images and successively copied on delayed ones.

Parameters derived from ROI analysis were the mean ^{99m}Tc-MIBI nodular uptake, pixel nodular number, mean ^{99m}Tc-MIBI background uptake and pixel background number.

The mean count in each ROI was determined, and early and late uptake results (ER and LR) were calculated as follows:

- **Early uptake result (ER) =** mean nodular ^{99m}Tc-MIBI uptake – mean background ^{99m}Tc-

MIBI uptake (early scans, 20 minutes after injection)

- **Late uptake result (LR) =** mean nodular ^{99m}Tc-MIBI uptake – mean background ^{99m}Tc-MIBI uptake (late scans, 60 minutes after injection)
- **The MIBI washout index (WOind)** was calculated as a percentage reduction value of the mean ^{99m}Tc-MIBI nodular uptake between early (+ 20 minutes) and late (+ 60 minutes) scans. **WOind** = [(LR/ER) X 100) – 100].

c.2.4) Surgery and pathology

Patients underwent total thyroidectomy on the basis of clinical, ultrasonographic and/or cytological criteria. A final histologic diagnosis was obtained after examination of permanent sections of the surgical specimens and served as the reference standard for establishing either the presence or the absence of thyroid malignant tumors.

d) Statistical analysis

Normality of distribution was assessed using histograms and the Shapiro-Wilk test. Quantitative variables were expressed as means (standard deviation) in the case of a normal distribution; otherwise, the median (interquartile range) was used. Categorical variables were expressed as numbers (percentage). Comparisons between the two groups were made using the Student's t-test for Gaussian continuous variables, the Mann-Whitney U test for non-Gaussian continuous variables and the Chi-squared test (or Fisher's exact test for expected cell frequency < 5) for categorical variables, as appropriate.

Receiver operating characteristic (ROC) analysis was also performed to determine the LR threshold below which malignant thyroid nodules could be ruled out. The area under the curve (AUC) was calculated to assess the overall accuracy of ^{99m}Tc-MIBI scintigraphy quantitative analysis.

We used the intraclass correlation coefficient (ICC) to assess inter-observer reproducibility of quantitative analysis; the ICC is rated good between 0.75 to 0.90 and excellent for values greater than 0.90.

Statistical analyses were performed using Graphpad-Prism 7.0 a software, and for ICC analysis, SPSS Version 23 (IBM SPSS Inc., Chicago, IL, USA) using a two-way-random-model. A two-sided p-value of <0.05 was considered statistically significant.

Results

The study design flow chart is presented in Figure 1. Seventy-one patients underwent a dual isotope scintigraphy. Of these patients, 5 were excluded because of the presence of a hot nodule, 16 because of indifferent nodules at ^{123}iodine scintigraphy, and 15 because no surgery was performed; therefore 35 patients were included in our study. Demographic, ultrasonographic, cytologic, scintigraphic and pathological data of the 35 patients were summarized in tables 1 and 2 in supplementary data.

a) Ultrasound

Most of the thyroid nodules were classified as EU-TIRADS 4 (n=18; 51.4%), ten (28.5%) were EU-TIRADS 3, and seven (20%) were EU-TIRADS 5 (Table 1).

b) Fine-needle aspiration cytology

FNAC was available for 34 (96.5%) patients, since one patient had an inaccessible nodule. The cytology results of 19 (55.8%) nodules were classified as Bethesda category IV, 5 (14.7%) were Bethesda III, and 10 (29.4%) were Bethesda I (Table 1).

c) Final histological diagnosis

The final histological diagnoses of the thyroidectomy specimens (our reference/gold standard) were 23 (65.7%) benign nodules and 12 (34.2%) malignant nodules (Table 2).

The malignant nodules were predominantly papillary thyroid carcinomas (n=6; 50%) and follicular variants of papillary thyroid carcinomas (n=3; 25%). Among the remaining malignant nodules, two were a Hürthle cell carcinomas and one was follicular thyroid carcinoma.

Of the 23 patients with benign nodules, 10 had colloid nodular goiters, 10 follicular adenomas, and 3 Hürthle cell adenomas.

d) Comparison between benign and malignant thyroid nodule groups

The mean age at nodule discovery was higher in the benign than in the malignant lesion group (mean age in years ±SD, respectively, 51.6 ±14.1 and 38.9 ± 15.7; p= 0.02). The mean TSH was not significantly different between the malignant and the benign nodule groups (mean of TSH in µIU/mL ± SD, respectively, 1.01 ± 0.31 and 1.29 ± 0.50; p=0.05) (Table 1).

d.1) Ultrasound

The mean largest diameter of the thyroid nodules was not different between the benign and malignant lesions (32 ± 13.2 mm and 38.5 ± 12.9 mm, respectively; p = 0.16). The majority of malignant (8/12; 66.6%) and benign (10/23; 43.4%) nodules were classified EU-TIRADS 4.

d.2) Fine-needle aspiration cytology

The prevalence of malignancy was 42.1% (8/19) in nodules with a Bethesda category IV cytology, 20% (1/5) in nodules with a Bethesda category III cytology and 27.3 % (3/11) in a Bethesda category I cytology.

Most nodules with malignant histology had a Bethesda category IV cytology result (n=8; 66.6%), 25% (3/12) were Bethesda I and one nodule was Bethesda III. Of the 23 nodules with benign histology, 11 (50%) had a Bethesda category IV cytology result, 4 (18.2%) were Bethesda III, and 7 (31.8%) were Bethesda I. FNAC had not been performed in one patient because of an inaccessible nodule.

d.3) Quantitative analysis of ^{99m}Tc-MIBI scintigraphy

Quantitative analysis of ^{99m}Tc-MIBI scintigraphy data showed that early (ER) and late (LR) ^{99m}Tc-MIBI uptake results were significantly higher in malignant than in benign nodules for both physicians (Table 3, Figures 2A and B). The difference was highly significant for the two nuclear medicine physicians concerning ER in malignant versus benign lesions. The difference was also highly significant in LR between malignant and benign thyroid nodules. We found the same results when comparing the means of ER and LR measurements of both physicians.

Inter-observer reproducibility between the two physicians was assessed by ICC. The ICC of ER was 0.86 (95% CI: [0.58-0.94]; p=0.0001) for benign nodules, and 0.92 (95% CI: [0.72-0.97]; p=0.0001) for malignant lesions. For LR, the ICC was 0.94 (95% CI: [0.85-0.97]; p=0.0001) for benign nodules and 0.85 (95% CI: [0.48-0.95] for malignant nodules (Figure 3).

There was no difference in the wash-out index between malignant and benign nodules (Table 3).

Using ROC curve analysis, the optimal cut-off for ER and LR corresponding to the highest accuracy for discriminating between benign and malignant thyroid lesions was set at 6.6 and 5.9, respectively. The AUC for ER was 0.78 (95% CI = 0.62 to 0.93; p= 0.006), and it was 0.78 (95% CI = 0.63 to 0.94; p= 0.005) for LR (Figures 2 D & E). Accordingly, 26 nodules had an ER greater than 6.6, of which 12 were histologically malignant and 14 had a benign histology. The remaining 9 nodules with an ER equal to or below this cut-off were all histologically benign. The sensitivity

and specificity of ^{99m}Tc-MIBI quantitative analysis for this cut-off were 100% and 39.1%, respectively. Positive predictive value, negative predictive value and accuracy were 46.1%, 100% and 60%, respectively.

Of the 20 nodules with an LR over 5.9, 12 had a malignant histology, whereas 8 were histologically benign (5 follicular adenomas, 2 Hürthle cell adenomas and one colloid nodular goiter). The remaining 15 nodules with an LR equal to or below this cut-off were all histologically benign (11 colloid nodular goiters, 4 follicular adenomas and one Hürthle cell adenoma). The sensitivity and specificity of ^{99m}Tc-MIBI quantitative analysis for this cut-off were 100% and 65.2%, respectively. Positive and negative predictive values and accuracy were 60%, 100% and 77.1%, respectively.

Therefore, LR had higher accuracy as well as a higher specificity and positive predictive value than ER for discriminating between benign and malignant thyroid lesions.

Discussion

In our series, the use of dual isotope ^{123}iodine/^{99m}Tc-MIBI scintigraphy allowed to exclude malignancy in euthyroid nodules with indeterminate or non-diagnostic cytology in a two steps evaluation. First, hot and warm nodules at ^{123}iodine evaluation were excluded since the ^{123}iodine uptake orientates in practice toward the benignity. Secondly, we used quantitative analysis of ^{99m}Tc-MIBI scintigraphy to further characterize cold nodules, since only 16% of them are reported to be malignant [19]. We thus found that the means of ^{99m}Tc-MIBI ER and LR were higher in malignant than in benign nodules. Moreover, LR had higher accuracy as well as a higher specificity and positive predictive value than ER for discriminating between benign and malignant thyroid lesions. Accordingly, using ROC curve the optimal LR cut-off that differentiated malignant from benign lesions was set at 5.9, with a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 100%, 65.2%, 60%, 100% and 77.1%, respectively. This

means that when the LR is < 5.9, benignity is certain. When the LR is >5.9, the risk of malignancy reaches 60%, and surgical thyroidectomy should be considered.

To our knowledge, this is the first report to describe the usefulness of LR measurement in the quantitative analysis of ^{99m}Tc-MIBI scintigraphy for ruling out malignancy of thyroid nodules with excellent sensitivity. Only two previous studies reported the usefulness of LR in quantitative analysis of ^{99m}Tc-MIBI scintigraphy to discriminate between malignant and benign nodules, but with a sensitivity of 61% and 83.3%, respectively [20,21].

In our study, all malignant nodules were correctly detected, but eight benign nodules were falsely positive (40%), of which six were follicular adenomas and two were Hürthle cell adenomas. All thyroid nodules with LR equal to or below this cut-off were histologically benign (10 colloid nodular goiter, 4 follicular adenomas and one Hürthle cell adenoma). Contrary to follicular adenomas, there was no colloid nodular goiter with an LR over the cut-off of 5.9, probably because the cell abundance is greater in follicular adenomas than in colloid nodular goiters. Similar results were reported by other authors [22].

Indeed, ^{123}Iodine/^{99m}Tc-MIBI scintigraphy with additional quantitative analysis seems to be an accurate method for evaluation of thyroid nodules with indeterminate or non-diagnostic cytology.

Moreover, in addition to the already known data, we report for the first time a very good inter-observer reproducibility for the quantitative analysis of ^{99m}Tc-MIBI scintigraphy for both ER and LR in malignant and benign nodules.

Various methods of quantitative ^{99m}Tc-MIBI analysis have been proposed through many studies [15,23,24]. Recently, the implementation of the wash-out index was found to be more accurate than the retention index in discriminating malignant from benign cytological indeterminate thyroid nodules, with an overall accuracy of 100% versus 62.5%, respectively [25].

In contrast with our study, given that the kinetic decrease in ^{99m}Tc-MIBI uptake (at 60 minutes) was similar in both malignant and benign lesions, there was no difference in the wash-out index between the two groups (p=0.94), and ^{99m}Tc-MIBI uptake in malignant lesions remained greater than in benign lesions. Hence, the use of the ^{99m}Tc-MIBI washout index in our study was not useful for differentiating benign from malignant thyroid nodules, as previously reported in another study including 43 patients with a solitary cold thyroid nodule, 9 of which were malignant and 34 benign [26].

The disparity between the results of previous studies can be explained, on the one hand, by the difference between protocols that were used, including the image acquisition times, and, on the other hand, by the fact that quantitative analysis is at least partially an instrument-dependent technique [27]. For these reasons, it is important to acknowledge that our results are specific to our center and that each department of nuclear medicine needs to set its own LR cut-off. Multicenter studies with a standard protocol are needed before being able to generalize and integrate ^{99m}Tc-MIBI scintigraphy in the guidelines of thyroid nodule evaluation.

Among several radioisotope imaging techniques, some recent studies have explored the role of Fluorine-18-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET)-computed tomography (CT) in discriminating benign from malignant thyroid nodules [28]. It was reported that ^{18}F-FDG PET/CT had a higher diagnostic performance in discriminating between benign and malignant indeterminate thyroid nodules compared to visual analysis of ^{99m}Tc-MIBI scintigraphy [29]. However, quantitative analysis of ^{99m}Tc-MIBI scintigraphy is more accurate than the visual analysis approach [25]. Also, a recent meta-analysis of eight studies reported that ^{18}F-FDG PET/CT had a moderate ability to correctly discriminate malignant from benign lesions [28].

Despite our encouraging results, some other limitations should be acknowledged. One of the limitations of ^{99m}Tc-MIBI scintigraphy is the impossibility of discriminating between Hürthle cell adenomas and Hürthle cell carcinomas, which has already been reported by several authors

343 [15,25,26,30–32]. The high uptake of ^{99m}Tc-MIBI by Hürthle cells is likely related to the negative
344 transmembrane mitochondrial electric potential [9,33].

345 Also, in our study only patients who had undergone surgery and had definitive histology of the
346 indeterminate cold thyroid nodule were included. Ideally, histological classification of all nodules,
347 from the initial cohort of 50 patients with a cold nodule, should be available to evaluate the
348 validity of a diagnostic approach. However, the majority of non-operated cold nodules remained
349 stable on US monitoring. Of these nodules, only one had an LR over the cut-off of 5.9, and
350 surgery was delayed due to other health issues.

351 The low number of patients in our study cohort could also be a limiting factor. Nevertheless,
352 similar results were reported by the same team in two series of 20 and 105 patients,
353 respectively, using quantitative analysis of ^{99m}Tc-MIBI [22,25].

354 To sum up, in euthyroid patients, quantitative analysis of ^{99m}Tc-MIBI scintigraphy could be
355 proposed as a second-line procedure for predicting malignant risk of solid thyroid nodules > 1cm,
356 with indeterminate or non-diagnostic cytology and cold at ^{123}iodine scintigraphy.

357 Nodules with LR uptake below or equal to the cut-off of 5.9, in which malignancy can be
358 excluded, support clinical and US follow-up. On the contrary, surgical treatment should be
359 discussed for thyroid nodules with LR uptake over the cut-off of 5.9 because of their high risk of
360 malignancy, reaching 60% in our study.

361

362 In conclusion, despite some limitations, our results suggest that dual isotope ^{123}Iodine/^{99m}Tc-
363 MIBI scintigraphy is an effective adjunctive pre-surgical method with a good interobserver
364 reproducibility, in combination with both US and FNAC, for ruling out malignancy of thyroid
365 nodules, thus reducing the number of unnecessary thyroidectomies. However, further
366 multicenter trial in larger series of patients will be necessary before being able to generalize and

integrate quantitative analysis of ^{99m}Tc-MIBI scintigraphy in the guidelines of thyroid nodules management.

Availability of data and materials: All data generated or analysed during this study are included in this published article (and its supplementary information files).

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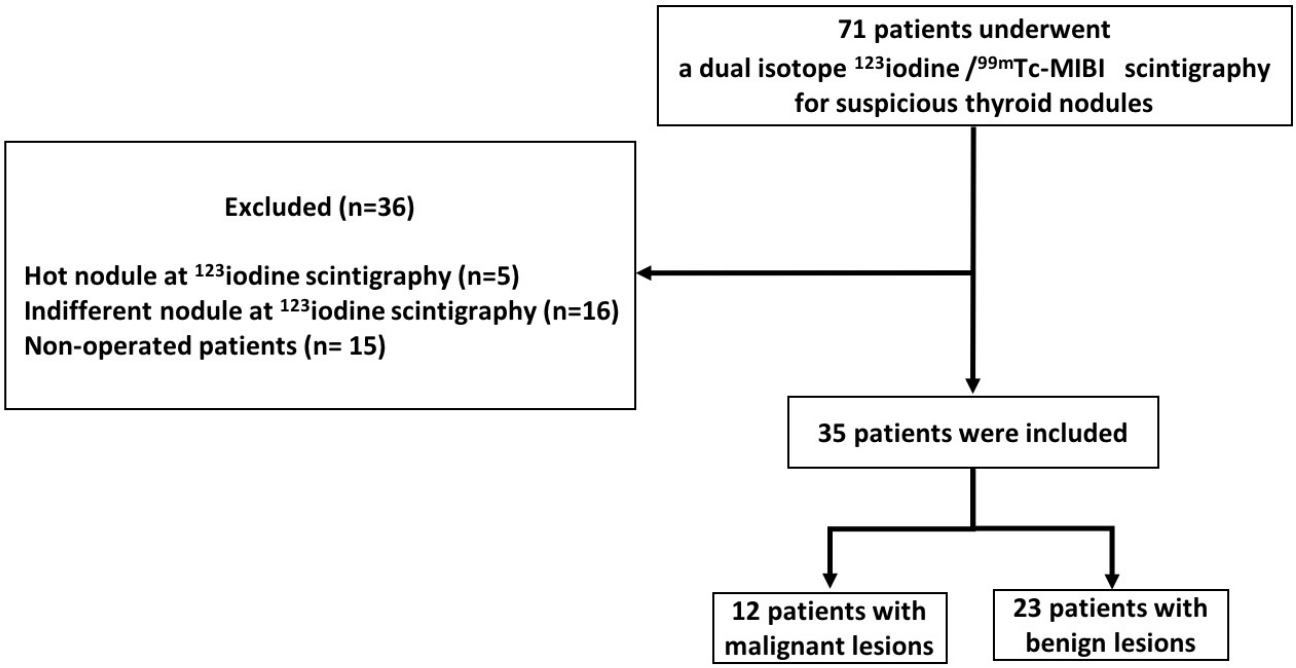

Figure 1. Flow chart of the study. 35 patients were operated and included

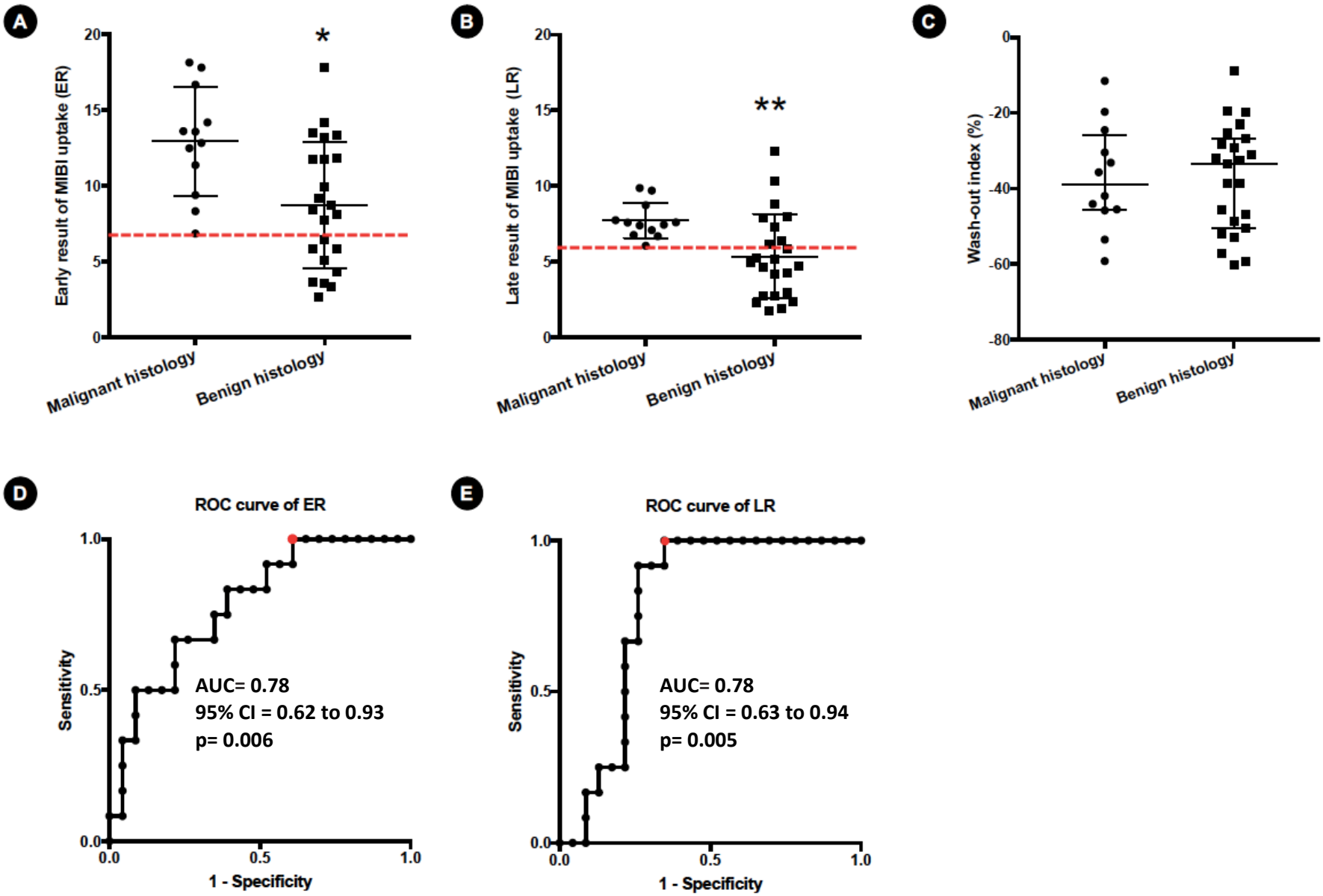
A
Early result of MIBI uptake (ER)
20
15
10
5
0
*
Malignant histology
Benign histology
B
Late result of MIBI uptake (LR)
20
15
10
5
0
**
Malignant histology
Benign histology
C
Wash-out index (%)
0
-20
-40
-60
-80
Malignant histology
Benign histology
D
ROC curve of ER
Sensitivity
1.0
0.5
0.0
0.0
0.5
1.0
1 - Specificity
AUC= 0.78
95% CI = 0.62 to 0.93
p= 0.006
E
ROC curve of LR
Sensitivity
1.0
0.5
0.0
0.0
0.5
1.0
1 - Specificity
AUC= 0.78
95% CI = 0.63 to 0.94
p= 0.005

Figure 2. Average measurements of (A) ER, (B) LR, (C) wash-out index in both malignant and benign groups. Dot and square plots depict ER and LR values from the 12 patients with malignant lesions, and the 23 patients with benign lesions, respectively; horizontal bars detail median and interquartile values. Red dashed lines represent the ER (6.6) and LR cut-offs (5.9). *****(p= 0.005), **(p=0.008).

(D), (E) Receiver operating characteristic (ROC) analysis of malignant versus benign lesions. Red dots depict optimal cut-off for ER (6.6) and LR (5.9). Area under the ROC curve (AUC) for ER = 0.78; (95% CI = 0.62 to 0.93; p= 0.006), and AUC for LR= 0.78; (95% CI = 0.63 to 0.94; p= 0.005). **For the ER cut-off:** Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy: **100%** (95% CI: [75.7-100]), **39.1%** (95% CI: [22.1-59.2]), **46.1%** (95% CI: [28.7-64.5]), **100%** (95% CI: [70-100]) and **60%**, respectively. **For the LR cut-off:** Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy: **100%** (95% CI: [75.7-100]), **65.2%** (95% CI: [44.8-81.1]), **60%** (95% CI: [38.6-78.1]), **100%** (95% CI: [79.6-100]) and **77.1%**, respectively.

Abbreviations: AUC: Area Under the Curve, 95% CI: 95% Confidence Interval. ER & LR: Early and late result of ^{99m}Tc-MIBI uptake within nodules, respectively.

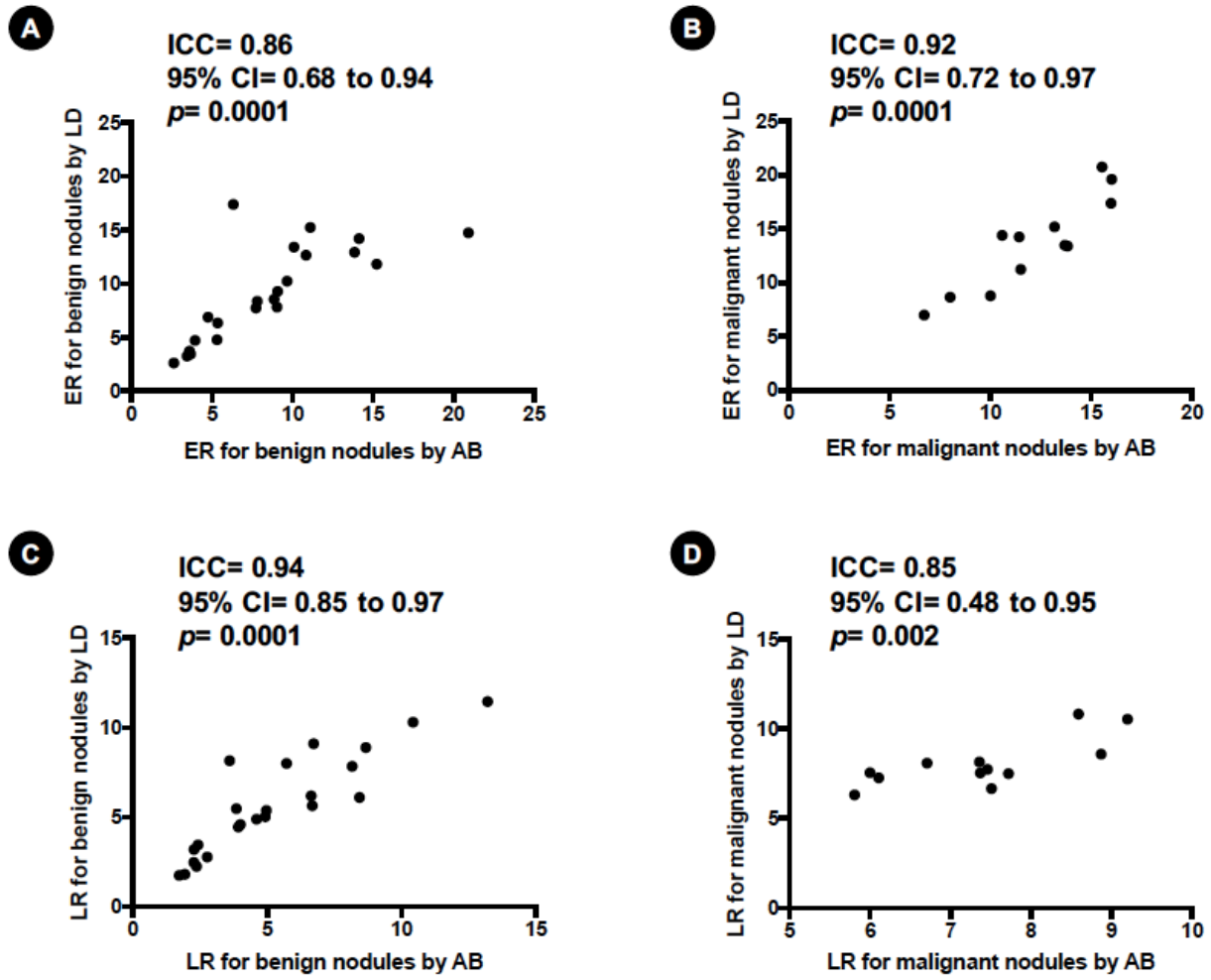

Figure 3. Intraclass correlation coefficients (ICC) between both physicians (AB and LD) of ER for benign (A), malignant lesions (B) and of LR for benign (C), malignant lesions (D).

Abbreviations: 95% CI: 95% Confidence Interval, ER & LR: Early and late result of ^{99m}Tc-MIBI uptake within nodule, respectively.

Table 1. Characteristics of patients (subdivided into those with benign and malignant histological findings after surgery).

Parameters	Malignant nodules (N = 12)	Benign nodules (N = 23)	N = 35	p-value
Age* (mean ± SD, years)	38.9 ± 15.7	51.6 ±14.1		**0.02**
Sex ratio	1 : 3	1 : 2.3		0.73
TSH (*mean ± SD;* µIU/mL)	1.29 ± 0.50	1.01 ± 0.31		0.05
Duration of follow-up since thyroid nodules discovery *(median (IQR),* months)	34.5 (25.7; 86.2)	23 (14; 56)		0.06
Duration of follow-up since MIBI scintigraphy *(median (IQR),* months)	6 (4; 9.2)	6 (5; 10)		0.52
Largest diameter of the nodule (mean ± SD, mm)	38.5 ± 12.9	32 ± 13.2		0.16
US classification				
EU-TIRADS 5 (n, %)	2 (16.6)	5 (21.7)	7 (20)	
EU-TIRADS 4 (n, %)	8 (66.6)	10 (43.4)	18 (51.4)	
EU-TIRADS 3 (n, %)	2 (16.6)	8 (34.7)	10 (28.5)	
FNAC				
Bethesda IV (n, %)	8 (66.6)	11 (50)	19 (55.9)	
Bethesda III (n, %)	1 (8.3)	4 (18.2)	5 (14.7)	
Bethesda I (n, %)	3 (25)	7 (31.8)	10 (29.4)	
Not done	0	1	1	

Abbreviations: IQR Interquartile, SD standard deviation, MIBI ^{99m}Tc-MIBI, US Ultrasound, FNAC Fine needle aspiration cytology.

*Age of the patient when thyroid nodule was discovered.

Table 2. Summary of final histological diagnosis and ^{99m}Tc-MIBI scintigraphy findings.

		^{99m}Tc-MIBI scintigraphy	
		Quantitative analysis n= 35	
Histology	**(n, %)**	LR cut-off > 5.9	LR cut-off ≤ 5.9
Benign			
Hürthle cell adenoma	3 (13%)	2	1
Follicular adenoma	10 (43.5%)	6	4
Colloid nodular goiter	10 (43.5%)	0	10
Total	**23 (100%)**	**8**	**15**
Malignant			
Hürthle cell carcinoma	2 (16.6%)	2	0
Papillary thyroid carcinoma	6 (50%)	6	0
Follicular variant of papillary thyroid carcinoma	3 (25%)	3	0
Follicular thyroid carcinoma	1 (8.3%)	1	0
Total	**12 (100%)**	**12**	**0**

Abbreviations: ER & LR: Early and late result of ^{99m}Tc-MIBI uptake within nodules, respectively.

Table 3. Summary of quantitative methods for ^{99m}Tc-MIBI scintigraphy findings.

	Malignant	Benign	P-value
	N = 12	N = 23	
Parameters			
MIBI scintigraphy analysis by AB			
ER (Mean ± SD (median, IQR))	12.2 ± 3 12.3 (10.1; 15.1)	8.4 ± 4.5 7.8 (4.7; 10.8)	**0.01**
LR (Mean ± SD (median, IQR))	7.4 ± 1.1 7.4 (6.2; 8.3)	5.2 ± 3 4.6 (2.4; 6.7)	**0.007**
Wash-out index (Mean ± SD (median, IQR))	-36 ± 12.4 -35.3 (-45.6; -25.3)	-37.3 ± 13.2 -38 (-46.3; -26.1)	0.77
MIBI scintigraphy analysis by LD			
ER (Mean ± SD (median, IQR))	13.6 ± 4.2 13.8 (9.4; 16.8)	8.9 ± 4.3 8.3 (4.7; 12.9)	**0.004**
LR (Mean ± SD (median, IQR))	8 ± 1.3 7.6 (7.3; 8.4)	5.4 ± 2.7 5 (3.2; 7.8)	**0.004**
Wash-out index (Mean ± SD (median, IQR))	-37.1 ± 15.3 40.1 (-47.4; -25.7)	-36.6 ± 16.1 -32.8 (-50.9; -27.4)	0.94
MIBI scintigraphy analysis (average: AB & LD)			
ER (Mean ± SD (median, IQR))	12.9 ± 3.5 13.2 (9.8; 16)	8.7 ± 4.1 8.4 (5; 11.8)	**0.005**
LR (Mean ± SD (median, IQR))	7.7 ± 1.1 7.5 (6.8; 8.4)	5.3 ± 2.7 4.9 (2.7;7.2)	**0.008**
Wash-out index (Mean ± SD (median, IQR))	-37 ± 13.9 (-38.8) (-45.7; -26)	-37.4 ± 14.2 -33.5 (-50.4; -26.8)	0.95

Abbreviations: SD Standard deviation, IQR Interquartile, MIBI ^{99m}Tc-MIBI, ER & LR : Early and late result of 99mTc-MIBI uptake within nodules, respectively.