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## Quantitative dual isotope $^{123}\text{I}$ / $^{99\text{m}}\text{Tc}$ -MIBI scintigraphy: A new approach to rule out malignancy in thyroid nodules

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### ► To cite this version:

Hamza Benderradji, Amandine Beron, Jean-Louis Wémeau, Bruno Carnaille, Laurent Delcroix, et al.. Quantitative dual isotope  $^{123}\text{I}$ / $^{99\text{m}}\text{Tc}$ -MIBI scintigraphy: A new approach to rule out malignancy in thyroid nodules. *Annales d'Endocrinologie = Annals of Endocrinology*, 2021, 82 (2), pp.83-91. 10.1016/j.ando.2021.03.003 . hal-04525532

**HAL Id: hal-04525532**

**<https://hal.science/hal-04525532v1>**

Submitted on 22 Jul 2024

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

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## 1 **Abstract**

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

5 **Methods:** 35 consecutive patients with thyroid nodules of indeterminate or non-  
6 diagnostic cytology and cold on <sup>123</sup>Iodine scintigraphy (10 Bethesda I, 24 Bethesda III-IV,  
7 1 in which cytology was impossible) underwent IMS between 2017 and 2019 with uptake  
8 quantification at two time points ahead of thyroidectomy: early and late. Images were  
9 analyzed by two blinded physicians

10 **Results:** 12 nodules were malignant and 23 benign on histopathology. Mean uptake  
11 values were lower in benign than in malignant nodules at both time points: early, 8.7±4.1  
12 versus 12.9±3.5 (p=0.005); and late, 5.3 ± 2.7 versus 7.7±1.1 (p=0.008). Inter-observer  
13 reproducibility was excellent. The intraclass correlation coefficient was 0.86 in benign and  
14 0.92 in malignant lesions on the early readings and 0.94 and 0.85 respectively on the late  
15 readings. The optimal cut-off to exclude malignancy on late reading was 5.9, with  
16 sensitivity, specificity, positive predictive value, negative predictive value and accuracy of  
17 respectively, 100%, 65.2%, 60%, 100% and 77.1%.

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

## 22 **Résumé**

23 **Contexte :** L'objectif de cette étude est d'évaluer l'apport de la Scintigraphie au <sup>99m</sup>Tc-  
24 MIBI combinée à l'Iode 123 (SMI) avec quantification de fixation, dans la discrimination  
25 bénin-malin des nodules thyroïdiens (NT) suspects.

26 **Patients et méthodes :** 35 patients consécutifs, porteurs d'un NT posant un problème  
27 diagnostique de malignité (10 NT Bethesda I, 24 NT Bethesda III-IV, 1 NT d'étude  
28 cytologique impossible) ont effectué une SMI avec mesure de la captation du traceur à  
29 deux temps d'acquisition précoce (TP) et tardif (TT) avant thyroïdectomie entre 2017 et  
30 2019. L'analyse des images de SMI a été réalisée en aveugle par deux praticiens.

31 **Résultats :** 12 nodules étaient malins et 23 bénins en analyse histologique. La moyenne  
32 (écart-type) de la mesure de captation du <sup>99m</sup>Tc-MIBI était plus élevée dans les cancers  
33 au TP (12,9 ± 3,5 vs. 8,7 ± 4,1 ; p=0,005) comme au TT (7,7 ± 1,1 vs. 5,3 ±  
34 2,7 ; p=0,008). La reproductibilité inter-observateurs était parfaite. Le coefficient de  
35 corrélation intra-classe était de 0,86 (lésions bénignes) et 0,92 (lésions malignes) pour le  
36 TP, et de 0,94 (lésions bénignes) et 0,85 (lésions malignes) pour le TT. Le seuil optimal  
37 de mesure de captation au TT pour l'exclusion de malignité a été fixé à 5,9. La  
38 sensibilité, spécificité, valeur prédictive positive, valeur prédictive négative, et précision  
39 de ce seuil étaient respectivement de 100%, 65,2%, 60%, 100% et 77,1% pour  
40 différencier un NT bénin et malin.

41 **Conclusion :** Malgré certaines limites, l'analyse quantitative de la scintigraphie  
42 thyroïdienne au <sup>99m</sup>Tc-MIBI est reproductible et pourrait contribuer à exclure la malignité  
43 des nodules thyroïdiens suspects, réduisant ainsi le nombre de thyroïdectomies inutiles  
44 lorsque la mesure de captation au TT est inférieure à 5,9.

45 **Keywords:** thyroid nodule, indeterminate or non-diagnostic cytology, <sup>99m</sup>Tc-MIBI  
46 scintigraphy, malignancy, surgery

47 **Mots clés :** nodule thyroïdien, cytologies indéterminées ou non diagnostiquées,  
48 scintigraphie au <sup>99m</sup>Tc-MIBI, malignité, chirurgie

53

54

## 55 **Background**

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

59 Both European and American guidelines suggest fine-needle aspiration cytology (FNAC) in  
60 combination with ultrasonography (US) for the evaluation of malignancy in thyroid nodules [2–  
61 4]. Published data suggest that FNAC has a sensitivity ranging from 65% to 98%, a specificity from  
62 72% to 100%, and an overall accuracy of 75% to 90%. Nevertheless, like any other test, FNAC has  
63 its limitations and diagnostic traps [5]. In 2–16% of cases, cytology is non-diagnostic and is  
64 classified Bethesda category I, i.e., with insufficient material for diagnosis. Moreover, in 5–20% of  
65 cases, it is not possible to discriminate between benign and malignant nodules because of an  
66 undetermined cytology, including atypia of undetermined significance/follicular lesion of  
67 undetermined significance, i.e., Bethesda category III, or because of follicular  
68 neoplasm/suspicious for follicular neoplasm, i.e., Bethesda category IV. The risk of malignancy  
69 varies from 5% to 10% for non-diagnostic cytology and from 6% to 40% for indeterminate  
70 cytology based on the 2017 revision of the Bethesda System for Reporting Thyroid Cytopathology  
71 [6]. About 60–95% of patients who underwent thyroidectomy because of non-diagnostic  
72 cytology or indeterminate cytology turned out to have a benign lesion at histological evaluation  
73 [7,8]. Hence, the use of a non-invasive approach is necessary.

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

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

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

92 In many published studies, <sup>99m</sup>Tc-MIBI scintigraphy was combined with <sup>99m</sup>Tc-pertechnetate,  
93 whereas <sup>123</sup>Iodine is more sensitive and allows better selection of cold thyroid nodules in  
94 comparison with <sup>99m</sup>Tc-pertechnetate [16].

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

99

## 100 **Patients and methods**

### 101 **a) Study design**

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

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

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

## 115 **b) Patients**

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

### 119 **b.1) Inclusion and exclusion criteria**

#### 120 **Inclusion criteria**

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

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

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

126

127 **Exclusion criteria**

128 **A.** Indifferent and hot nodules at  $^{123}\text{I}$  evaluation on dual isotope scintigraphy.

129 **B.** Non-operated patients.

130 **c) Study outcomes**

131 All patients underwent physical examination, biological thyroid assays (TSH, FT4, FT3),  
132 thyroid US, FNAC (except one patient with an inaccessible nodule) and dual isotope  
133  $^{123}\text{I}$ iodine/ $^{99\text{m}}\text{Tc}$ -MIBI scintigraphy.

134 **c.2.1) Ultrasonography**

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

140 **c.2.2) Fine-needle aspiration cytology**

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

144 **c.2.3) Dual isotope scintigraphy**

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

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

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

155 In <sup>123</sup>Iodine scintigraphy, thyroid nodules were assessed as being either 1) hyperfunctioning,  
156 i.e., hot (uptake in the nodule is greater than uptake in normal thyroid tissue); 2) indifferent, i.e.,  
157 warm (uptake in the nodule is equal to uptake in normal thyroid tissue); or 3) hypofunctioning,  
158 i.e., cold (uptake in the nodule is less than uptake in normal thyroid tissue).

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

162

### 163 **Quantitative analysis**

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

169 Parameters derived from ROI analysis were the mean <sup>99m</sup>Tc-MIBI nodular uptake, pixel  
170 nodular number, mean <sup>99m</sup>Tc-MIBI background uptake and pixel background number.

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

173 • **Early uptake result (ER)** = mean nodular <sup>99m</sup>Tc-MIBI uptake – mean background <sup>99m</sup>Tc-

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

180

#### 181 **c.2.4) Surgery and pathology**

182 Patients underwent total thyroidectomy on the basis of clinical, ultrasonographic and/or

183 cytological criteria. A final histologic diagnosis was obtained after examination of permanent

184 sections of the surgical specimens and served as the reference standard for establishing either

185 the presence or the absence of thyroid malignant tumors.

#### 186 **d) Statistical analysis**

187 Normality of distribution was assessed using histograms and the Shapiro-Wilk test.

188 Quantitative variables were expressed as means (standard deviation) in the case of a normal

189 distribution; otherwise, the median (interquartile range) was used. Categorical variables were

190 expressed as numbers (percentage). Comparisons between the two groups were made using the

191 Student's t-test for Gaussian continuous variables, the Mann-Whitney U test for non-Gaussian

192 continuous variables and the Chi-squared test (or Fisher's exact test for expected cell

193 frequency < 5) for categorical variables, as appropriate.

194 Receiver operating characteristic (ROC) analysis was also performed to determine the LR

195 threshold below which malignant thyroid nodules could be ruled out. The area under the curve

196 (AUC) was calculated to assess the overall accuracy of  $^{99m}\text{Tc}$ -MIBI scintigraphy quantitative

197 analysis.

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

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

204

## 205 **Results**

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

212

### 213 **a) Ultrasound**

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

216

### 217 **b) Fine-needle aspiration cytology**

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

221

222 **c) Final histological diagnosis**

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

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

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

230 **d) Comparison between benign and malignant thyroid nodule groups**

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

235

236 **d.1) Ultrasound**

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

240

241 **d.2) Fine-needle aspiration cytology**

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

245 Most nodules with malignant histology had a Bethesda category IV cytology result (n=8; 66.6%),  
246 25% (3/12) were Bethesda I and one nodule was Bethesda III. Of the 23 nodules with benign  
247 histology, 11 (50%) had a Bethesda category IV cytology result, 4 (18.2%) were Bethesda III, and 7  
248 (31.8%) were Bethesda I. FNAC had not been performed in one patient because of an  
249 inaccessible nodule.

250

### 251 **d.3) Quantitative analysis of <sup>99m</sup>Tc-MIBI scintigraphy**

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

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

262 There was no difference in the wash-out index between malignant and benign nodules (Table  
263 3).

264 Using ROC curve analysis, the optimal cut-off for ER and LR corresponding to the highest  
265 accuracy for discriminating between benign and malignant thyroid lesions was set at 6.6 and 5.9,  
266 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 =  
267 0.63 to 0.94; p= 0.005) for LR (Figures 2 D & E). Accordingly, 26 nodules had an ER greater than  
268 6.6, of which 12 were histologically malignant and 14 had a benign histology. The remaining 9  
269 nodules with an ER equal to or below this cut-off were all histologically benign. The sensitivity

270 and specificity of <sup>99m</sup>Tc-MIBI quantitative analysis for this cut-off were 100% and 39.1%,  
271 respectively. Positive predictive value, negative predictive value and accuracy were 46.1%, 100%  
272 and 60%, respectively.

273 Of the 20 nodules with an LR over 5.9, 12 had a malignant histology, whereas 8 were  
274 histologically benign (5 follicular adenomas, 2 Hürthle cell adenomas and one colloid nodular  
275 goiter). The remaining 15 nodules with an LR equal to or below this cut-off were all histologically  
276 benign (11 colloid nodular goiters, 4 follicular adenomas and one Hürthle cell adenoma). The  
277 sensitivity and specificity of <sup>99m</sup>Tc-MIBI quantitative analysis for this cut-off were 100% and  
278 65.2%, respectively. Positive and negative predictive values and accuracy were 60%, 100% and  
279 77.1%, respectively.

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

## 282 **Discussion**

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

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

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

301 In our study, all malignant nodules were correctly detected, but eight benign nodules were  
302 falsely positive (40%), of which six were follicular adenomas and two were Hürthle cell  
303 adenomas. All thyroid nodules with LR equal to or below this cut-off were histologically benign  
304 (10 colloid nodular goiter, 4 follicular adenomas and one Hürthle cell adenoma). Contrary to  
305 follicular adenomas, there was no colloid nodular goiter with an LR over the cut-off of 5.9,  
306 probably because the cell abundance is greater in follicular adenomas than in colloid nodular  
307 goiters. Similar results were reported by other authors [22].

308 Indeed, <sup>123</sup>Iodine/<sup>99m</sup>Tc-MIBI scintigraphy with additional quantitative analysis seems to be an  
309 accurate method for evaluation of thyroid nodules with indeterminate or non-diagnostic  
310 cytology.

311 Moreover, in addition to the already known data, we report for the first time a very good  
312 inter-observer reproducibility for the quantitative analysis of <sup>99m</sup>Tc-MIBI scintigraphy for both ER  
313 and LR in malignant and benign nodules.

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

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

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

332 Among several radioisotope imaging techniques, some recent studies have explored the role  
333 of Fluorine-18-fluorodeoxyglucose ( $^{18}\text{F}$ -FDG) positron emission tomography (PET)-computed  
334 tomography (CT) in discriminating benign from malignant thyroid nodules [28]. It was reported  
335 that  $^{18}\text{F}$ -FDG PET/CT had a higher diagnostic performance in discriminating between benign and  
336 malignant indeterminate thyroid nodules compared to visual analysis of  $^{99m}\text{Tc}$ -MIBI scintigraphy  
337 [29]. However, quantitative analysis of  $^{99m}\text{Tc}$ -MIBI scintigraphy is more accurate than the visual  
338 analysis approach [25]. Also, a recent meta-analysis of eight studies reported that  $^{18}\text{F}$ -FDG  
339 PET/CT had a moderate ability to correctly discriminate malignant from benign lesions [28].

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

343 [15,25,26,30–32]. The high uptake of <sup>99m</sup>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 <sup>99m</sup>Tc-MIBI [22,25].

354 To sum up, in euthyroid patients, quantitative analysis of <sup>99m</sup>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 <sup>123</sup>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 <sup>123</sup>Iodine/<sup>99m</sup>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

367 integrate quantitative analysis of <sup>99m</sup>Tc-MIBI scintigraphy in the guidelines of thyroid nodules  
368 management.

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370

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

373 **Acknowledgments:** The authors would like to thank endocrinologists from the Nord and Pas-de-  
374 Calais departments who participated in patient recruitment for the study. The authors also thank  
375 Janet Ratiu for the English review of the manuscript.

376 **Declaration of interest:** The authors declare that there is no conflict of interest that could be  
377 perceived as prejudicing the impartiality of the research reported.

378 **Funding:** This research did not receive any specific grant from any funding agency in the public,  
379 commercial or nonprofit sector.

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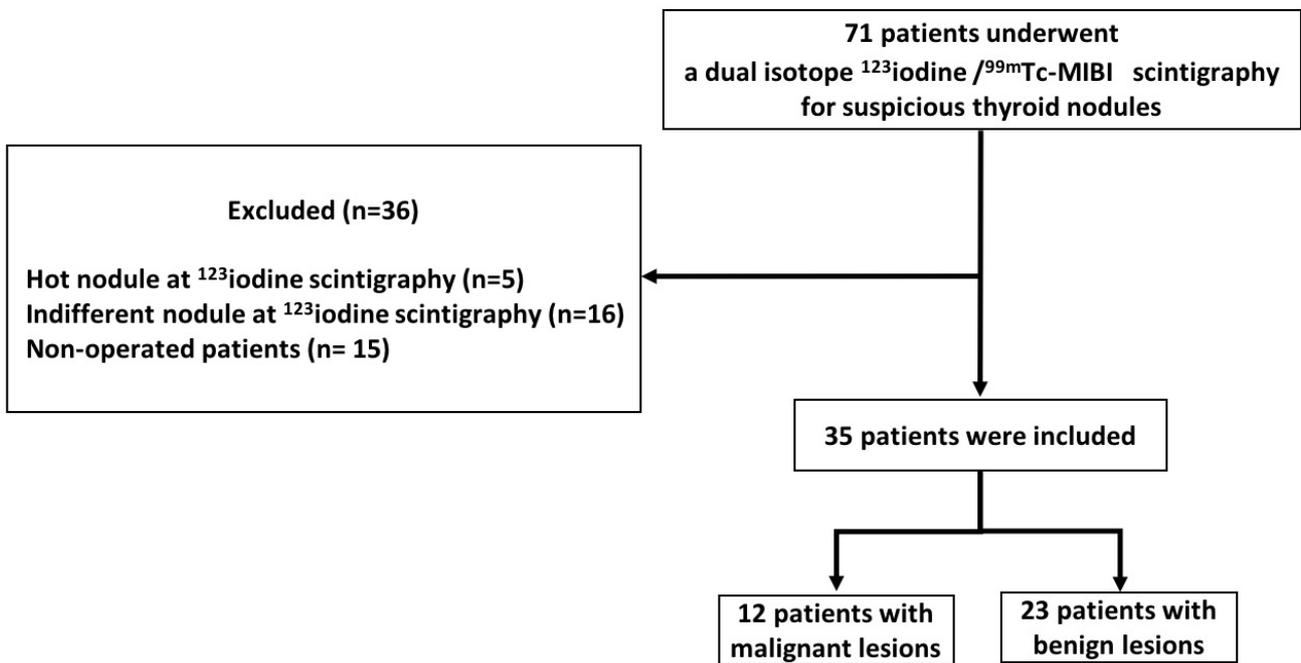
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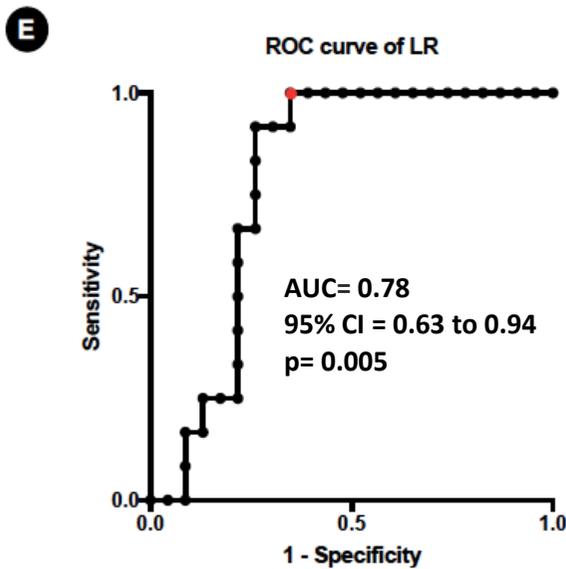
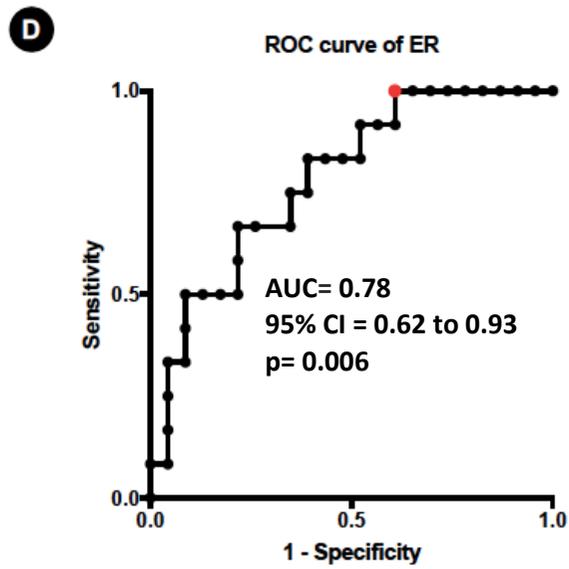
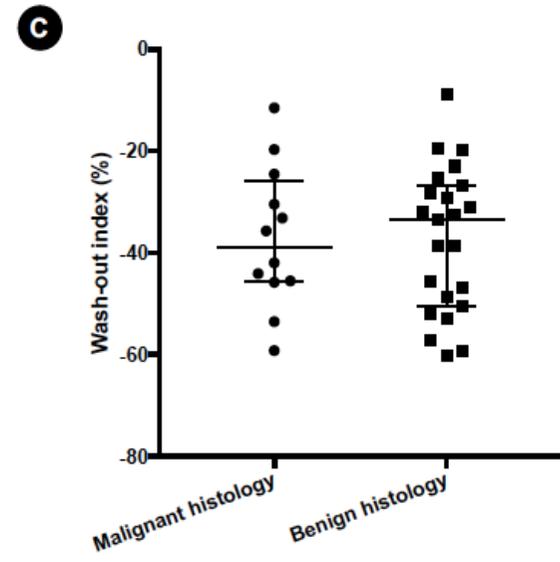
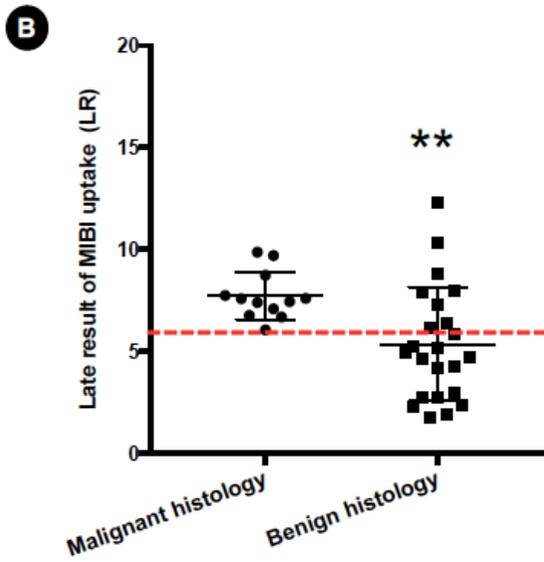
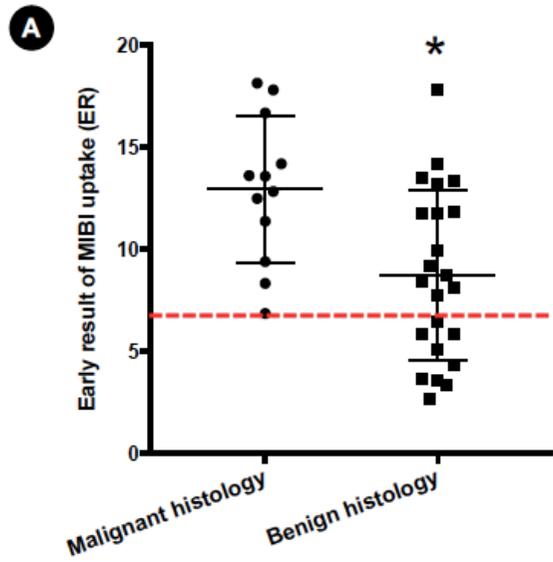
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**Figure 1. Flow chart of the study.** 35 patients were operated and included



**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 <sup>99m</sup>Tc-MIBI uptake within nodules, respectively.*

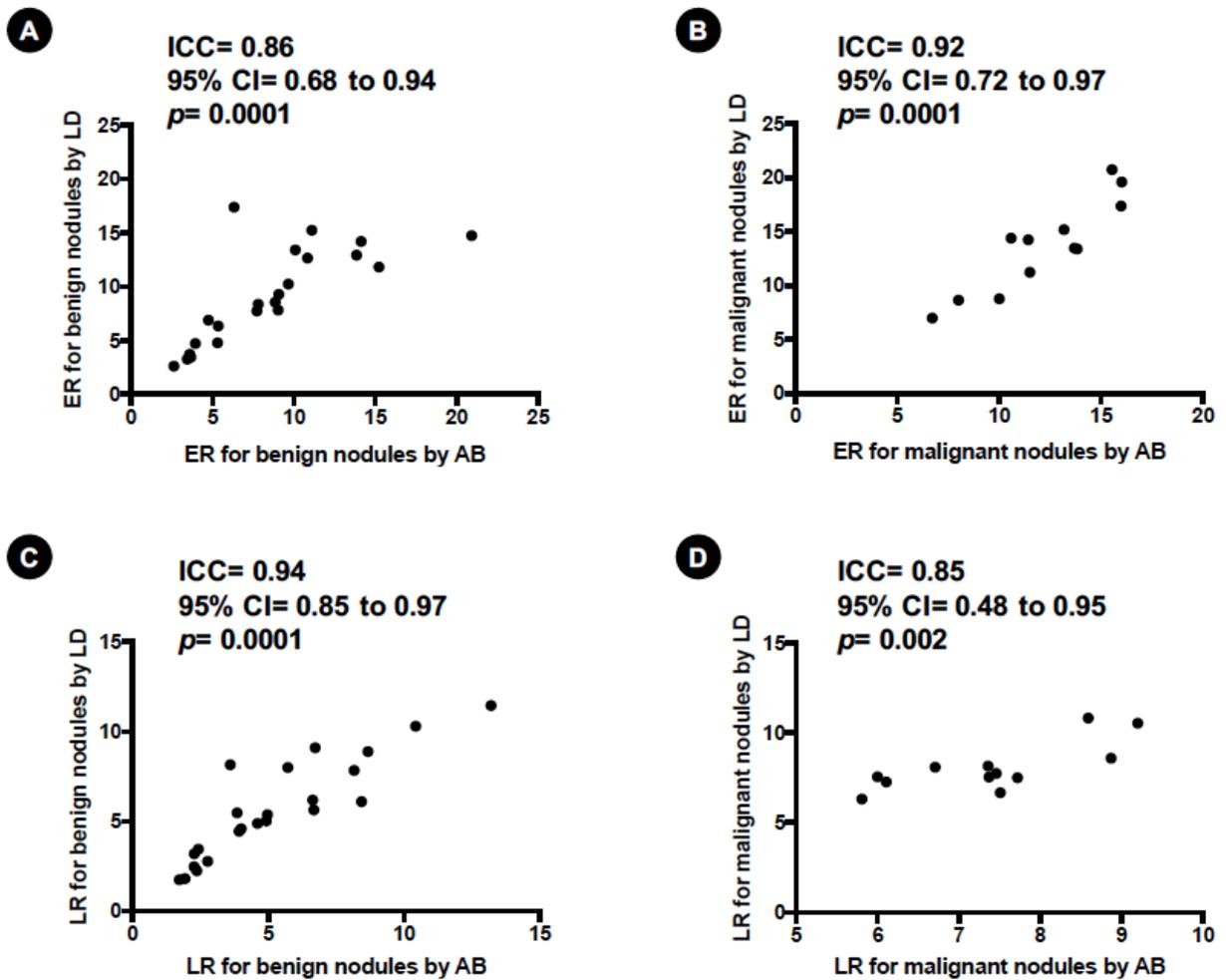


Figure 3. Intraclass correlation coefficients (ICC) between both physicians (AB and LD) of ER for benign (A), malignant lesions (B) and of LR for benign (C), malignant lesions (D).

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

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

Parameters	Malignant (N = 12)	Benign (N = 23)	nodules N = 35	p-value
Age* (mean $\pm$ SD, years)	38.9 $\pm$ 15.7	51.6 $\pm$ 14.1		<b>0.02</b>
Sex ratio	1 : 3	1 : 2.3		0.73
TSH (mean $\pm$ SD; $\mu$ IU/mL)	1.29 $\pm$ 0.50	1.01 $\pm$ 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 $\pm$ SD, mm)	38.5 $\pm$ 12.9	32 $\pm$ 13.2		0.16
<b>US classification</b>				
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)	
<b>FNAC</b>				
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 <sup>99m</sup>Tc-MIBI scintigraphy findings.

Histology	(n, %)	<sup>99m</sup> Tc-MIBI scintigraphy	
		Quantitative analysis n= 35	
		LR cut-off > 5.9	LR cut-off ≤ 5.9
<b>Benign</b>			
Hürthle cell adenoma	3 (13%)	2	1
Follicular adenoma	10 (43.5%)	6	4
Colloid nodular goiter	10 (43.5%)	0	10
<b>Total</b>	<b>23 (100%)</b>	<b>8</b>	<b>15</b>
<b>Malignant</b>			
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
<b>Total</b>	<b>12 (100%)</b>	<b>12</b>	<b>0</b>

Abbreviations: ER & LR: Early and late result of <sup>99m</sup>Tc-MIBI uptake within nodules, respectively.

**Table 3.** Summary of quantitative methods for <sup>99m</sup>Tc-MIBI scintigraphy findings.

	Malignant	Benign	P-value
	N = 12	N = 23	
<b>Parameters</b>			
<b>MIBI scintigraphy analysis by AB</b>			
ER (Mean ± SD (median, IQR))	12.2 ± 3 12.3 (10.1; 15.1)	8.4 ± 4.5 7.8 (4.7; 10.8)	<b>0.01</b>
LR (Mean ± SD (median, IQR))	7.4 ± 1.1 7.4 (6.2; 8.3)	5.2 ± 3 4.6 (2.4; 6.7)	<b>0.007</b>
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
<b>MIBI scintigraphy analysis by LD</b>			
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)	<b>0.004</b>
LR (Mean ± SD (median, IQR))	8 ± 1.3 7.6 (7.3; 8.4)	5.4 ± 2.7 5 (3.2; 7.8)	<b>0.004</b>
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
<b>MIBI scintigraphy analysis (average: AB &amp; LD)</b>			
ER (Mean ± SD (median, IQR))	12.9 ± 3.5 13.2 (9.8; 16)	8.7 ± 4.1 8.4 (5; 11.8)	<b>0.005</b>
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)	<b>0.008</b>
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 <sup>99m</sup>Tc-MIBI, ER & LR : Early and late result of <sup>99m</sup>Tc-MIBI uptake within nodules, respectively.