The Italian Consensus for the Classification and Reporting of Thyroid Cytology: Cytohistologic and molecular correlations on 37,371 nodules from a single institution

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BACKGROUND: The Italian Consensus for the Classification and Reporting of Thyroid Cytology (ICCRTC) includes six diagnostic categories (TIR 1/1C, TIR 2, TIR 3A, TIR 3B, TIR 4, and TIR 5), each indicating a different risk of malignancy. The objective of this monocentric retrospective study was to evaluate the distribution of the ICCRTC classes at the authors' institution and assess their cytohistologic correlations. **METHODS:** The authors retrospectively collected 37,371 consecutive cytologic reports of thyroid nodules and described the clinical–pathologic features of the different cytologic categories. The cytologic diagnoses also were compared with histologic outcomes in a subset of patients. **RESULTS:** The cytologic classes were distributed as follows: nondiagnostic, 15.6%; benign, 66.5%; low-risk indeterminate, 10% (TIR 3A); high-risk indeterminate, 3.5% (TIR 3B); suspicious, 1.7%; and malignant, 2.6%. According to histology, the risk of malignancy was very high in the nondiagnostic category (29.8%), with young male patients more exposed to malignancy, and it was relatively high among benign (7.8%) and indeterminate nodules (32.5% in TIR 3A; 52.1% in TIR 3B), mainly because of the high prevalence of follicular architecture in malignant tumors. On histology, the malignancy rates were 92.4% and 99.3% for the suspicious and malignant categories, respectively; aggressive variants of papillary thyroid carcinoma were mostly diagnosed in these categories. **CONCLUSIONS:** In this series, nondiagnostic nodules showed high prevalence and, surprisingly, high malignancy rates. Malignant tumors with follicular architecture represented a diagnostic pitfall in benign and indeterminate nodules. The suspicious and malignant categories had high specificity for malignancy. Importantly, the ICCRTC had high reliability for identifying preoperatively aggressive histotypes of thyroid carcinoma. ***Cancer Cytopathol* 2022;130:899-912.** © 2022 The Authors. *Cancer Cytopathology* published by Wiley Periodicals LLC on behalf of American Cancer Society This is an open accessarticle under the terms of the Creative Commons Attribution-NonCommercial License, which permits use,distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

KEY WORDS: cytology; fine-needle aspiration; histology; thyroid cancer; thyroid nodules.

INTRODUCTION

Fine-needle aspiration (FNA) cytology is the most accurate and cost-effective procedure for evaluating thyroid nodules. FNA in association with thyroid function testing and ultrasonography is the initial procedure in the clinical management of thyroid nodules.[1] The goal of cytologic thyroid nodule evaluation is to drive the selection of patients for surgery or for conservative management. Moreover, it helps to determine the type, frequency, and length of follow-up.[2] Most of the classification systems for reporting cytologic results of aspirated thyroid nodules provide different diagnostic categories; each category implies a different risk of

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malignancy (ROM).[3–5] In our practice, thyroid FNAs are routinely diagnosed according to the Italian Consensus for the Classification and Reporting of Thyroid Cytology (ICCRTC).[6] The ICCRTC system is basically comparable with other internationally recognized systems—in particular, the Bethesda System for Reporting Thyroid Cytology (Bethesda) and the UK Royal College of Pathologists (UK RCPath) classification for thyroid FNA—although some differences in terminology are evident, especially in the indeterminate categories.[7,8] The expression *indeterminate cytology* commonly refers to the ICCRTC TIR 3A and TIR 3B classifications and the Bethesda class III or IV, which are associated with malignancy rates that vary from 10% to 40%. In the presence of an indeterminate result, the possible clinical actions include the repetition of FNA, molecular testing, and/or diagnostic lobectomy.[9,10]

The objective of this study was to define the distribution of cytologic diagnoses in a large consecutive series of thyroid nodules evaluated at the same institution. These cytologic diagnoses are correlated to the histologic outcome and molecular profile of the subset of nodules from patients who underwent to surgery.

MATERIALS AND METHODS

Patient cohorts

In this retrospective, single-center, observational study, we collected all consecutive thyroid FNA reports and their clinical data from the Surgical Pathology database of the University Hospital of Pisa between 2015 and 2020. All patients had undergone FNA cytology at the Unit of Endocrinology, and a subset of patients had been submitted to surgery at the Unit of Endocrine Surgery of the University Hospital of Pisa. Both informed and surgical consent were obtained before the operation.

Thyroid FNA was performed by a team of five skilled endocrinologists under ultrasonographic guidance using a 25-gauge needle (with at least two passes for each nodule). All cytologic specimens were stained with Papanicolaou, evaluated by expert thyroid cytopathologists, and classified according to the ICCRTC.[6] This thyroid FNA classification encompasses the following six diagnostic categories (Table 1)[1-13]:

TIR 1

The *nondiagnostic category* includes specimens consisting of blood and lacks both colloid and an adequate number of follicular cells; for solid nodules, a sample can be labeled adequate only when at least six groups of 10 well preserved epithelial cells are detected. This category includes aspirates with abundant colloid and macrophages consistent with cysts (TIR 1C).

TIR 2

The *nonmalignant/benign category* includes goiter, hyperplastic nodules, thyroiditis, and other non-neoplastic conditions. According to clinical data, samples with

TABLE 1. Italian Consensus for the Classification and Reporting of Thyroid Cytology and comparison with other classification systems

ICCRTC	Bethesda (Cibas & Ali, 2017[11])	UK RCPath (Lobo 2011[12])	JTA (Kakudo 2014[13])
TIR 1. Nondiagnostic	I. Nondiagnostic or unsatisfactory	Thy1. Nondiagnostic for cytologic diagnosis	1. Inadequate (nondiagnostic)
TIR 1C. Nondiagnostic–cystic		Thy1c. Nondiagnostic for cytologic diagnosis—cystic lesion	
TIR 2. Nonmalignant/benign	II. Benign	Thy2. Nonneoplastic Thy2c. Nonneoplastic—cystic lesion	2. Normal or benign
TIR 3A. Low-risk indeterminate lesion	III. Atypia of undetermined significance or follicular lesion of undetermined significance	Thy3a. Neoplasm possible—atypia/nondiagnostic	3. Indeterminate
TIR 3B. High-risk indeterminate lesion	IV. Follicular neoplasm or suspicious for a follicular neoplasm	Thy3f. Neoplasm possible, suggesting follicular neoplasm	A. Follicular neoplasm A-1. Favor benign A-2. Borderline A-3. Favor malignant B. Others
TIR 4. Suspicious of malignancy	V. Suspicious for malignancy	Thy4. Suspicious of malignancy	4. Malignancy suspected
TIR 5. Malignant	VI. Malignant	Thy5. Malignant	5. Malignancy

Abbreviations: Bethesda, The Bethesda System for Reporting Thyroid Cytology; ICCRCT, Italian Consensus for the Classification and Reporting of Thyroid Cytology; JTA, the Japan Thyroid Association reporting system; UK RCPath, the United Kingdom Royal College of Pathologists reporting system.

abundant colloid and poor cellularity obtained from cysts or spongiform nodules identified on ultrasound or in samples with intense lymphocytic cellularity from patients with clinical evidence of Hashimoto thyroiditis can be considered TIR 2.

TIR 3A

The *low-risk indeterminate lesion category* is characterized by increased cellularity with microfollicular structures and poor colloid; the overall appearance of microfollicles is not sufficient to suspect a follicular neoplasm. This subcategory includes samples with some degree of cytologic or architectural alteration, probably because of technical artifacts, not otherwise specified.

TIR 3B

The *high-risk indeterminate lesion category* includes specimens that show high cellularity with monotonous microfollicular and/or trabecular arrangement and scant or absent colloid; the suspicion of a follicular neoplasm is high. This subcategory encompasses samples with nuclear alterations suggestive of papillary thyroid carcinoma (PTC) but too mild to label a sample as TIR 4. Moreover, samples composed exclusively or almost exclusively of Hurthle cells are included in this category, and the cell type is mentioned in the report.

TIR 4

The *suspicious for malignancy category* includes samples with only some features of malignancy but for which a definitive diagnosis of malignancy cannot be made.

TIR 5

The *malignant category* includes cases with a definitive cytologic diagnosis of malignancy. Representative images of the ICCRTC cytologic categories are shown in Figure 1.

The lowest and highest risk cytologic categories (TIR1–TIR2 and TIR4–TIR5, respectively) proposed by the ICCRTC system resemble those of the most popular reporting systems of thyroid cytology (Bethesda,[11] UK RCPath,[12] and the Japan Thyroid Association [JTA][13] reporting systems), as shown in Table 1. The most striking differences across the reporting systems concern the criteria of adequacy necessary to define a sample as benign. Because the ROM (<1%) is similar in benign cytology and in cysts, the UK RCPath system[12] and the JTA system[13] clearly define the cyst fluid–only samples as benign and not as nondiagnostic, although the revised Bethesda system[11] reinforces many exceptions to the adequacy of the cytologic samples. Regarding the indeterminate categories, the subdivision into two groups exists in all of the above-mentioned reporting systems. The ICCRTC system proposes two subcategories of indeterminate lesions, closely resembling indeterminate group A and indeterminate group B of the JTA system. In both of these systems, the presence of equivocal or too mild nuclear atypia of papillary carcinoma endorses the label of indeterminate nodule at high ROM. Notably, only the Japanese reporting system encourages the further optional subclassification of indeterminate group A into three subcategories (A-1, favor benign; A-2, borderline; and A-3 favor malignant).

In the case of multiple aspirated nodules per patient, the nodule with the highest cytologic category was taken into account. Whenever nodules of the same patient had the same cytologic diagnosis, the greater in size was considered.

In our center, mutational analysis is not performed on all the indeterminate nodules but is carried out in clinically selected nodules and upon the endocrinologist's request. For DNA extraction, manual dissection of areas of interest was conducted on cytologic smears; DNA purification was performed by using a commercial kit (Qiamp DNA Mini Kit; Qiagen). Molecular testing was done by using an allele-specific real-time polymerase chain reaction commercial kit investigating the most frequent molecular alterations detectable in thyroid cytology: *BRAF* codons 600 and 601; *NRAS* codons 12, 13, and 61; *HRAS* codons 12, 13, and 61; and *KRAS* codons 12, 13, and 61 (EasyThyroid; Diatech Pharmacogenetics).

Surgical thyroid specimens obtained from thyroidectomy or hemithyroidectomy were diagnosed by pathologists with expertise in endocrine pathology. The histologic diagnosis was made according to the fourth edition of the *World Health Organization Classification of Endocrine Organs*.[14]

Statistical analyses

Continuous variables were analyzed using the Welch *t*-test. The Pearson χ^2 test with an analysis of residuals was used for categorical variables. The Fisher exact test was

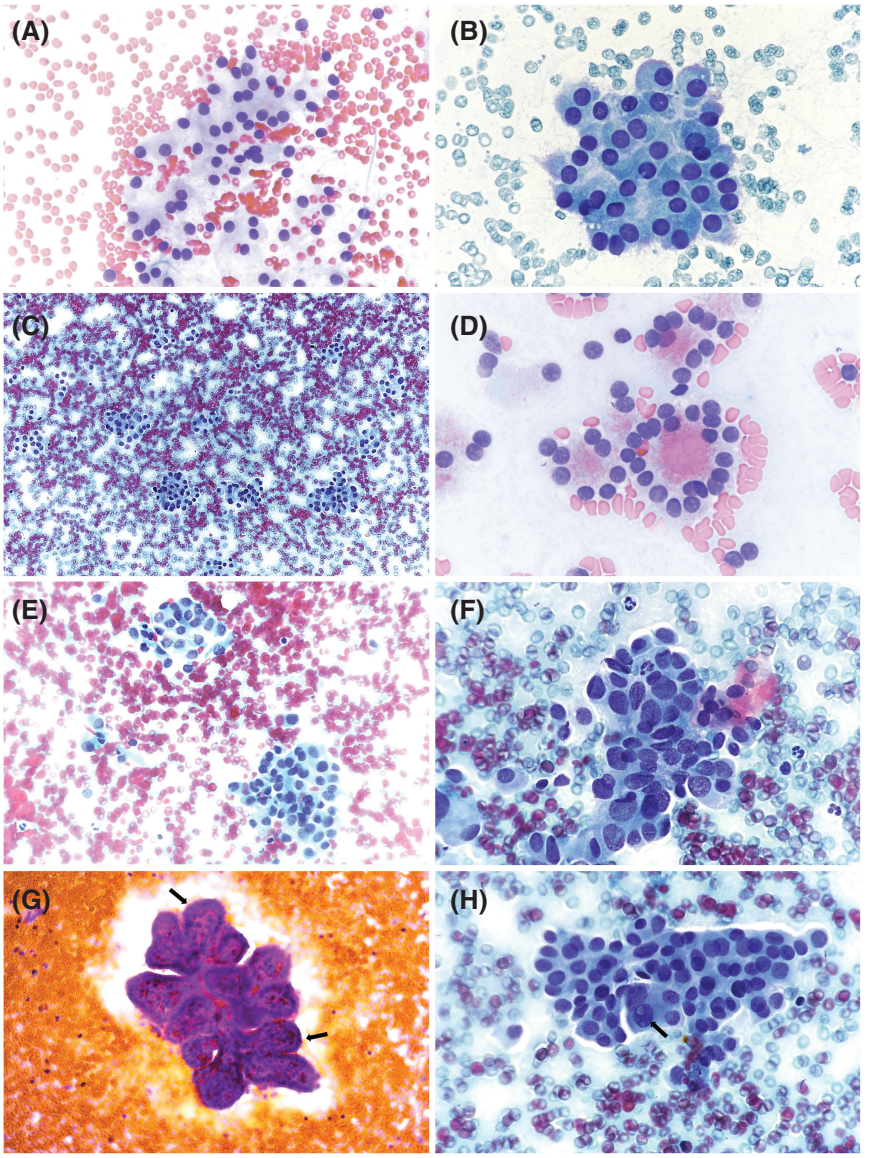

Figure 1. Representative cytologic images of different thyroid fine-needle aspirations classified according to the Italian Consensus for the Classification and Reporting of Thyroid Cytology (ICCRTC) system. (A,B) Nonmalignant/benign (TIR 2). Regular sheets of follicular cells without nuclear atypia (Papanicolaou staining; original magnification ×40 and ×60 in A and B, respectively). (C,D) Low-risk indeterminate lesion (TIR 3A). This cytologic sample at different magnification shows well formed and regular microfollicles with colloid inside and follicular cells with small, rounded, hyperchromatic nuclei (Papanicolaou staining; original magnification ×20 and ×60 in C and D, respectively). (E) High-risk indeterminate lesion (TIR 3B). Follicular cells are organized in small, irregular sheets and show mild nuclear alterations, i.e., elongation, overlapping, and powdery chromatin (Papanicolaou staining; original magnification ×40). (F) Suspicious for malignancy (TIR 4). This cluster is composed of follicular cells with evident nuclear alterations but is not sufficient to make a diagnosis of malignancy, i.e., enlarged nuclei with irregular nuclear membrane and nuclear grooves (Papanicolaou staining; original magnification ×60). (G,H) Malignant (TIR 5). Papillary thyroid carcinoma at different magnifications: a well formed neoplastic papillary structure is shown (arrows); at higher magnification, the typical nuclear alterations of papillary thyroid carcinoma are shown, including enlarged, oval nuclei with nuclear pseudoinclusions (arrow; Papanicolaou staining; original magnification ×20 and ×60 in G and H, respectively).

run whenever appropriate. All p values $< .05$ were considered significant. Positive and negative predictive values, sensitivity, and specificity with related 95% confidence intervals (95% CIs) were computed by 2000 bootstrap resampling and following the procedures of the pROC package, version 1.18.0. All analyses were performed in R environment (version 4.0.2; https://www.r-project.org/; accessed January 27, 2022).

RESULTS

The final study population consisted of 37,371 thyroid nodules in 25,903 patients (19,733 females and 6170 males; female-to-male ratio, 3.2:1.0). The patients ranged in age from 4 to 93 years (mean ± SD, 54 ± 14 years). The mean nodule size was 24.0 ± 12.2 mm. The distribution of cytologic diagnoses in the six diagnostic categories was as follows (Table 2): 3981 nodules were TIR 1 (nondiagnostic; 10.7%), and 1836 were TIR 1C (nondiagnostic–cystic; 4.9%); 24,862 nodules were TIR 2 (nonmalignant/benign; 66.5%); 3747 nodules were TIR 3A (low-risk indeterminate lesions; 10.0%), and were 1326 TIR 3B (high-risk indeterminate lesions; 3.5%); 644 nodules were TIR 4 (suspicious for malignancy; 1.7%); and 975 nodules were TIR 5 (malignant; 2.6%). According to the cytologic categories, the mean patient age varied from 55.8 years in the TIR 2 category to 45.5 years in the TIR 5 category ($p < .001$; Figure 2A). The female-to-male ratio ranged from 3.9 in the benign group to 1.9 in the malignant group (Figure 2B). The mean size of the aspirated nodules varied from 17.8 mm in the TIR 5 category to 26.9 mm in the TIR 1C category ($p < .001$; Figure 2C). Finally, the occurrence of a single nodule in the thyroid gland was more frequent in the highest cytologic categories (TIR 3B, TIR 4, and TIR 5: 70.2%, 71.1%, and 73.1%, respectively) compared with the low-risk cytologic categories (above all, the TIR 2 category, 40%; $p < .001$; Figure 2D).

TABLE 2. Patient demographics and nodule characteristics

Variable	No. of patients (%)
Patients	25,903 (100.0)
Sex	
Female	19,733 (76.2)
Male	6170 (23.8)
Age: Mean ± SD, years	54 ± 14
Thyroid nodules	37,371
Single nodule	17,391 (67.1)
Multiple nodules	19,980 (32.9)
Size: Mean ± SD, mm	24.2 ± 12.2
Diagnostic category on FNA	
TIR 1	3981 (10.7)
TIR 1C	1836 (4.9)
TIR 2	24,862 (66.5)
TIR 3A	3747 (10.0)
TIR 3B	1326 (3.5)
TIR 4	644 (1.7)
TIR 5	975 (2.6)

Abbreviation: FNA, fine-needle aspiration.

Surgical follow-up

In total, 4908 patients (18.9%) had undergone surgery, which included either hemithyroidectomy or total thyroidectomy (Table 3). The surgical rate was high (≥79.8%) in the cytologic categories TIR 3B and above, and most patients underwent total thyroidectomy. Conversely, the surgical rate was low in the cytologic categories TIR 1C and TIR 2 (5.8% and 6.5%, respectively). Notably, the surgical rate of the nondiagnostic category TIR 1 (10.4%) was higher than that of the TIR 1C and TIR 2 categories. In most cases, patients underwent total thyroidectomy, even in the presence of benign cytology, probably for the presence of multiple nodules in both lobes of the thyroid gland.

Cytologic–histologic correlation

The malignant rate on histology was very high in the TIR 4 and TIR 5 nodules at 92.9% and 99.3%, respectively (Table 4), whereas the rate of malignancy in the intermediate categories varied from 32.5% for TIR 3A to 52.1% for TIR 3B. As shown in Table 4, noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTPs) were considered separately. Most histologically proven NIFTPs had an indeterminate cytologic diagnosis (TIR 3A or TIR 3B). The main performance indicators of cytologic evaluation are reported in Table 5. Table 6 provides the distribution of the most important thyroid malignancies according to cytologic category. In each cytologic group, the most prevalent histotypes were micro-PTC (i.e., PTC ≤1 cm) and PTC. Figure 3 illustrates the percentage of the most frequent variants of PTC according to the cytologic category.

Nondiagnostic category: TIR 1 and TIR 1C

In total, 220 (10.4%) of the 2112 patients who had nondiagnostic cytology (TIR 1) and 68 (5.8%) of the 1168 patients who had nondiagnostic–cystic cytology (TIR 1C) were surgically treated. The surgical approach was driven by specific clinical indications (i.e., suspicious ultrasonographic characteristics of the nodule or compressive symptoms), as indicated in Table 7 (data were available for 181 of 288 patients who underwent surgery). Notably, surgical follow-up revealed a malignancy in 86 patients (29.8%), including 22 micro-PTCs, 45 PTCs, five follicular thyroid carcinomas (FTCs), four anaplastic thyroid carcinomas, seven medullary thyroid carcinomas, and three other malignant lesions (Table 6). The mean

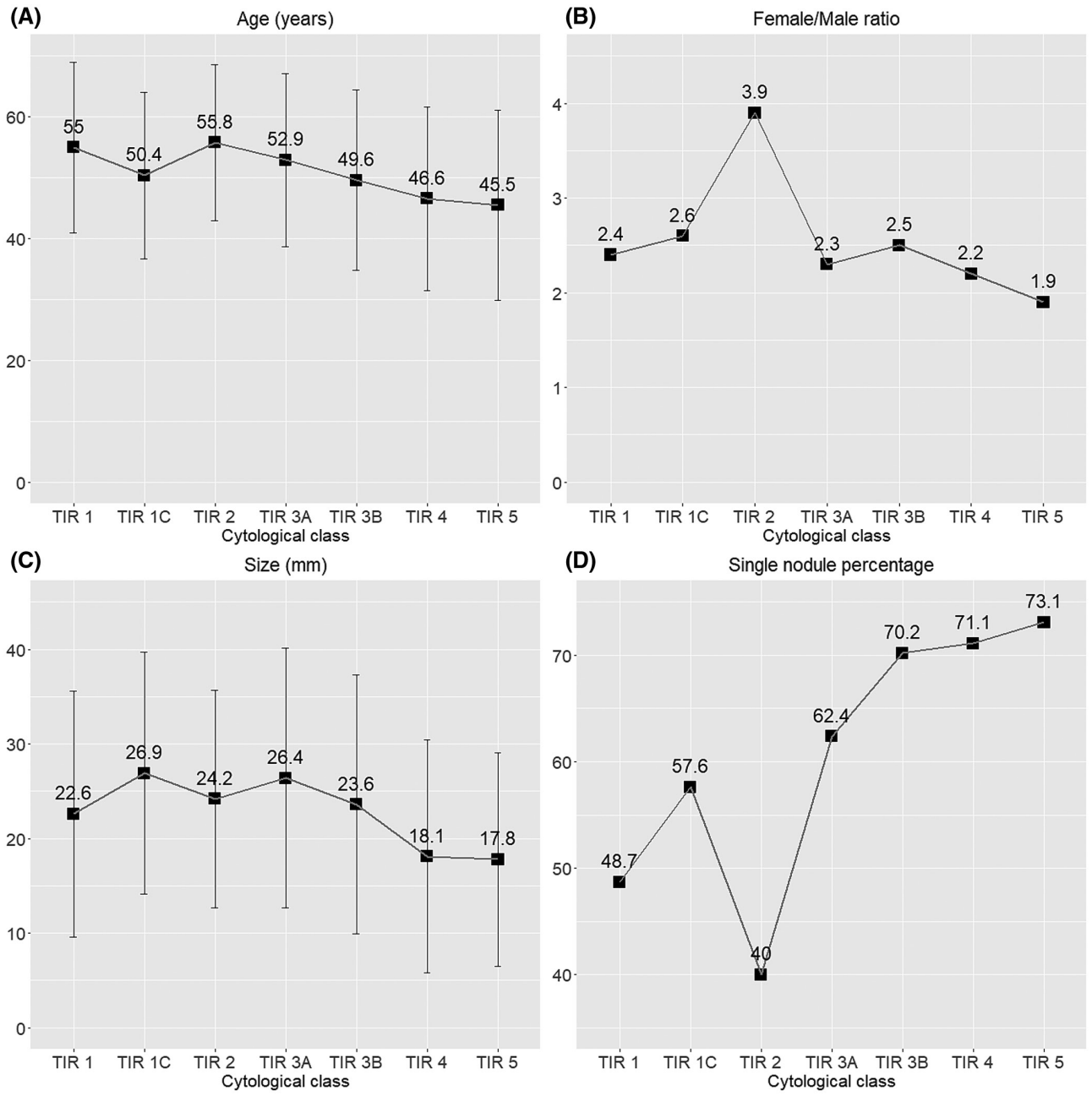

Figure 2. Trends in patients' demographics and nodule characteristics according to cytologic class

age of patients who had malignant histology was younger than that of patients who had TIR 1 with benign histology (45.6 vs. 52.6 years; $p = .0005$). Moreover, the ROM more than doubled in males with nondiagnostic cytology compared with females.

Nonmalignant/benign: TIR 2

Among patients who had benign cytology, 1064 underwent surgery (6.5%). A finding of malignancy on histologic examination occurred in 83 of these patients (7.8%), with a prevalence of PTC, which was diagnosed in 49 of the 83 patients (59.1%). In light of the malignant histologic outcome, FNA slides from these 49 PTCs were independently reviewed by three pathologists. In 47 cases, none of the pathologists noticed the presence of papillary nuclear characteristics. In one case, cell representativeness was limited, and the sample was deemed nondiagnostic (TIR 1). In one case, pathologists collegially agreed on a

TABLE 3. Surgical outcome of patients

Diagnostic category on FNA	No. of patients	No. of patients with surgical outcome (%)	Total thyroidectomy, no. (%)
TIR 1	2112	220 (10.4)	162 (73.6)
TIR 1C	1168	68 (5.8)	40 (58.8)
TIR 2	16,401	1064 (6.5)	933 (87.7)
TIR 3A	3449	1183 (34.3)	911 (77.0)
TIR 3B	1238	988 (79.8)	830 (84.0)
TIR 4	612	565 (92.3)	523 (92.6)
TIR 5	923	820 (88.8)	768 (93.7)
Total	25,903	4908 (18.9)	4167 (84.9)

Abbreviation: FNA, fine-needle aspiration.

TABLE 4. Cytologic–histologic correlation

	Diagnostic category on FNA, no. of patients (%)					
Histologic diagnosis	TIR 1/TIR 1C, *N* = 288	TIR 2, *N* = 1064	TIR 3A, *N* = 1183	TIR 3B, *N* = 998	TIR 4, *N* = 565	TIR 5, *N* = 820
Benign	198 (68.8)	959 (90.2)	728 (61.6)	448 (45.3)	37 (6.6)	5 (0.6)
Malignant	86 (29.8)	83 (7.8)	385 (32.5)	515 (52.1)	525 (92.9)	814 (99.3)
NIFTP	4 (1.4)	22 (2.0)	70 (5.9)	35 (3.6)	3 (0.5)	1 (0.1)

Abbreviations: FNA, fine-needle aspiration; NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features.

TIR 3B diagnosis because of the presence of mild nuclear atypia (nuclear enlargement and clearing); the nodule histology was classic PTC.

Indeterminate lesions: TIR 3A and TIR 3B

Approximately 34% of patients who had TIR 3A nodules underwent surgical control, with a prevalence of total thyroidectomy. The malignant rate at histology was 32.5% (385 of 1183 patients). The large majority of malignancies were PTCs (289 of 385 patients; 75%), with a high prevalence of the follicular variant PTC (Figure 2 and Table 8). Conversely, in patients who had a cytologic diagnosis of TIR 3B, the surgical rate was very high (79.8%). At histology, malignancy was identified in 515 patients (malignant rate, 52.1%). Among the TIR 3B nodules, the most frequent malignant lesion was PTC (322 of 515 patients; 62.3%), mainly the of follicular variant (Figure 2 and Table 8).

Suspicious of malignancy and malignant: TIR4–TIR5

Most patients who had a cytologic diagnosis of suspicious or malignant nodules (TIR 4 or TIR 5) underwent surgery, with surgical rates of 92.6% and 93.7%, respectively. Malignancy was detected or confirmed at histology in 92.9% and 99.3% of patients who had TIR 4 and TIR 5 nodules, respectively. The large majority of tumors was micro-PTCs and PTCs, with a prevalence of the classical variant of PTC (Figure 3 and Table 8). The tall cell variant of PTC was almost exclusively detected in patients who had a cytologic diagnosis of TIR 4 or TIR 5 (Figure 3 and Table 8)

TABLE 5. Performance indicators of cytology

Indicator	Median (95% CI), %
TIR 5 specificity	99.5 (99.0–99.9)
TIR 5 PPV	99.4 (98.9–99.9)
TIR 4 specificity	96.3 (95.0–97.4)
TIR 4 PPV	93.5 (91.3–95.3)
TIR 3B specificity	68.2 (65.7–70.6)
TIR 3B PPV	55.1 (53.1–57.3)
TIR 3A specificity	56.8 (54.5–59.2)
TIR 3A PPV	38.4 (36.9–40.0)
TIR 2 NPV	90.1 (88.3–91.8)

Abbreviations: PPV, positive predictive value; NPV, negative predictive value.

Molecular analysis on FNAs

Mutation analysis was performed in a selected series of patients (835 of 25,903 patients; 3.2%), mostly in indeterminate nodules (12.6% of TIR 3A lesions and 12.7% of TIR 3B lesions). Mutation analysis failed in 27 of 835 patients (3.2%) because of insufficient DNA input or inhibition of the polymerase chain reaction enzyme.

TABLE 6. Cytologic–histologic correlation of malignant lesions

Histologic diagnosis	Diagnostic category on FNA, no. of patients (%)					
	TIR 1/TIR 1C, *N* = 86	TIR 2, *N* = 83	TIR 3A, *N* = 385	TIR 3B, *N* = 515	TIR 4, *N* = 525	TIR 5, *N* = 814
Micro-PTC, *n* = 421	22 (25.6)	13 (15.7)	22 (5.7)	68 (13.2)	145 (27.6)	151 (18.6)
PTC, n = 1626	45 (52.3)	49 (59.1)	289 (75.0)	322 (62.3)	330 (62.9)	591 (72.6)
FTC, *n* = 166	5 (5.8)	10 (12.0)	59 (15.3)	85 (16.5)	7 (1.3)	0 (0.0)
HCC, *n* = 26	0 (0.0)	0 (0.0)	1 (0.3)	21 (4.1)	4 (0.8)	0 (0.0)
PDTC, *n* = 47	0 (0.0)	1 (1.2)	4 (1.0)	15 (2.9)	14 (2.7)	13 (1.6)
ATC, *n* = 14	4 (4.7)	0 (0.0)	1 (0.3)	0 (0.0)	3 (0.6)	6 (0.7)
MTC, *n* = 86	7 (8.1)	6 (7.2)	6 (1.6)	1 (0.2)	17 (3.2)	49 (6.0)
Other malignancies, *n* = 22	3 (3.5)	4 (4.8)	3 (0.8)	3 (0.6)	5 (0.9)	4 (0.5)

Abbreviations: ATC, anaplastic thyroid carcinoma; FTC, follicular thyroid carcinoma; HCC, Hurthle cell carcinoma; MTC, medullary thyroid carcinoma; PDTC, poorly differentiated thyroid carcinoma; PTC, papillary thyroid carcinoma.

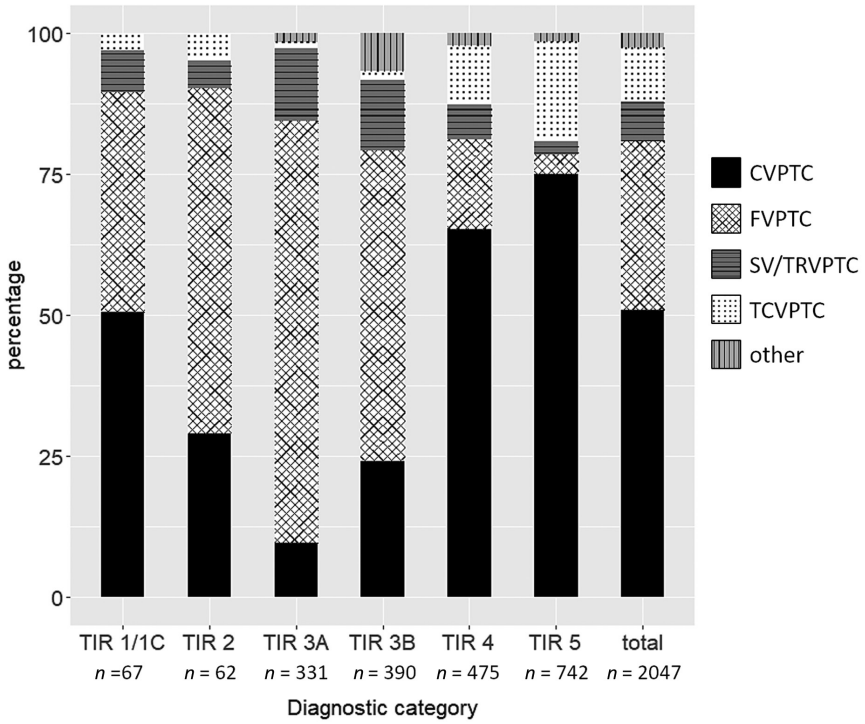

Figure 3. Distribution of papillary thyroid carcinoma variants according to the cytologic diagnostic category. CV indicates classic variant; FV, follicular variant; PTC, papillary thyroid carcinoma; SV, solid variant; TCV, tall cell variant; TRVPTC, trabecular variant.

TABLE 7. Reasons driving surgery of TIR 1/TIR 1C nodules[a]

Description	No. (%)
Repeated TIR 1 cytology	55 (30.4)
Multinodular goiter	36 (19.9)
Suspicious FNA performed in other centers	26 (14.3)
Homolateral lymphadenopathy	17 (9.4)
Compressive symptoms with or without tracheal deviation	16 (8.8)
Highly suspicious US features or large nodule size	11 (6.1)
High serum calcitonin	9 (5.0)
Clinical thyroiditis	9 (5.0)

Abbreviations: FNA, fine-needle aspiration; US, ultrasonography.
[a]Data were available for 181 of 288 nodules.

Overall, molecular testing was successfully performed in 808 samples, which were distributed as shown in Table 9. The prevalence of mutations increased with the cytologic category, ranging from 12% in benign nodules to 73% in malignant nodules ($p < .0001$). *RAS*-like mutations (*NRAS*, *HRAS*, *KRAS*, and *BRAF* K601E) were detected in 127 of 180 mutation-positive nodules, representing >70% of all mutated cases, according to the predominant presence of nodules characterized by a follicular pattern. *BRAF* V600E mutations were more frequent in suspicious

TABLE 8. Distribution of the variants of papillary thyroid carcinoma according to cytologic diagnostic category

	Cytology class, no. of nodules							
PTC variant	TIR 1	TIR 1C	TIR 2	TIR 3A	TIR 3B	TIR 4	TIR 5	Total
CV	30	4	18	30	94	310	557	1043
FV	22	4	38	233	215	76	25	613
SV/TRV	5	0	3	40	49	29	18	144
TCV	2	0	3	3	6	50	132	196
Other	0	0	0	5	26	10	10	51
Total	59	8	62	311	390	475	742	2047

Abbreviations: CV, classical variant; FV, follicular variant; PTC, papillary thyroid carcinoma; SV, solid variant; TCV, tall cell variant; TRV, trabecular variant.

TABLE 9. Molecular testing results according to cytologic classes[a]

		No./total no. (%)				
Diagnostic category on FNA	No./total no. (%)	Analysis failed	Mutation negative	Mutation positive	*BRAF* V600E	*RAS*-like
TIR 1 and TIR 1C	27/3280 (0.8)	13/27 (48.2)	11/27 (40.7)	3/27 (11.1)	1/27 (3.7)	2/27 (7.4)
TIR 2	133/16,401 (0.8)	4/133 (3.0)	113/133 (85.0)	16/133 (12.0)	2/133 (1.5)	14/133 (10.5)
TIR 3	434/3449 (12.6)	9/434 (2.1)	343/434 (79.0)	82/434 (18.9)	3/434 (0.7)	79/434 (18.2)
TIR 3B	157/1238 (12.7)	0 (0.0)	123/157 (78.3)	34/157 (21.7)	7/157 (4.5)	27/157 (17.2)
TIR 4	47/612 (7.7)	0 (0.0)	29/47 (61.7)	18/47 (38.3)	13/47 (27.7)	5/47 (10.6)
TIR 5	37/923 (4.0)	1/37 (2.7)	9/37 (24.3)	27/37 (73.0)	27/37 (73)	0 (0.0)
Total	835/25,903 (3.2)	27/835 (3.2)	628/835 (75.2)	180/835 (21.6)	53/835 (6.4)	127/835 (15.2)

Abbreviation: FNA, fine-needle aspiration.
[a]The numbers of samples submitted to molecular analysis on the total numbers of nodules in each diagnostic category are reported. Mutant nodules have been further divided into *BRAF* V600E-like and *RAS*-like. All percentages refer to the total number of nodules undergoing molecular testing.

and malignant nodules, whereas they were identified in only 1.5% of TIR 2 nodules, 0.7% of TIR 3A nodules, and 4.5% of TIR 3B nodules.

Histologic outcomes were available for 256 nodules that underwent had presurgical molecular testing, as reported in Table 10. In the TIR 3A nodules, *RAS*-like mutations were present in 10 of 37 benign nodules (27%), in 4 of 8 NIFTPs (50%), and in 15 of 36 PTCs (41.7%). In the TIR 3B nodules, *RAS*-like mutations were absent in benign nodules and were detected in four of five NIFTPs (80%) and in 12 of 37 PTCs (32.4%). Considering only the *RAS*-like mutations in the surgical cohort, these were preoperatively detected in 64 nodules. Overall, 15 were benign (23.4%), 10 were NIFTPs (15.6%), and the remaining 39 were malignant at histology (61%). In the TIR 3A nodules, mutation testing showed a positive predictive value of 65.6% (95% CI, 53.3%–80.6%) and a negative predictive value of 55.1% (95% CI, 47.8%–64.4%); in the TIR 3B nodules, the positive predictive value was 100%, and the negative predictive value was 47.4% (95% CI, 42.2%–54.0%).

DISCUSSION

We collected and analyzed data from a large series of consecutive thyroid FNAs, consisting of more than 37,000 nodules in more than 25,000 patients who were evaluated in a single institution, to assess the distribution of the cytologic categories and their correlation with histologic outcomes.

Our study confirmed some of the most common and well known demographic aspects of thyroid nodules. Thyroid nodules are more commonly observed in women, and the large majority of these appear benign at cytologic examination.[15] The occurrence of benign cytology was more frequent in patients older than 55 years, whereas thyroid nodules with suspicious or frankly malignant cytology were more frequently observed in the younger population.[16–18] We previously reported that the rate of benign cytology was higher in elderly patients than in younger patients, probably because of the more frequent use of imaging techniques in elderly patients to investigate other concurrent morbidities.[19]

TABLE 10. Surgical outcome of 256 nodules with presurgical molecular analysis available

	No. (%)						
	Surgery performed		Histologic diagnosis				
Diagnostic category on FNA	No	Yes	Benign	NIFTP	PTC	FTC	PDTC
TIR 1, *n* = 27	23 (85)	4 (15)	3 (75)		1 (25)		
		Wild type	1				
		RAS-like	2				
		BRAF V600E			1		
		Failed					
TIR 2, *n* = 133	113 (85)	20 (15)	11 (55)	2 (10)	6 (30)	1 (5)	
		Wild type	7		2	1	
		RAS-like	3	2	3		
		BRAF V600E			1		
		Failed	1				
TIR 3A, *n* = 434	348 (80)	86 (20)	37 (43)	8 (9)	36 (42)	5 (6)	
		Wild type	27	4	19	3	
		RAS-like	10	4	15	2	
		BRAF V600E			2		
		Failed					
TIR 3, *n* = 157	74 (47)	83 (53)	27 (33)	5 (6)	37 (45)	12 (14)	2 (2)
		Wild type	27	1	19	9	2
		RAS-like		4	12	3	
		BRAF V600E			6		
		Failed					
TIR 4, *n* = 47	12 (25)	35 (75)	7 (20)	0 (0)	25 (71)	1 (3)	2 (6)
		Wild type	7		11	1	2
		RAS-like			4		
		BRAF V600E			10		
		Failed					
TIR 5, *n* = 37	9 (24)	28 (76)	0 (0)	0 (0)	28 (100)	0 (0)	0 (0)
		Wild type			6		
		RAS-like					
		BRAF V600E			22		
		Failed					

Abbreviations: FNA, fine-needle aspiration; FTC, follicular thyroid carcinoma; NIFTP, noninvasive follicular neoplasm with papillary-like nuclear features; PDTC, poorly differentiated thyroid carcinoma; PTC, papillary thyroid carcinoma.

The current results indicate an overall rate of nondiagnostic cytology of 10.7%. This percentage is in line with the rates reported in some series[5,18] but is higher compared with other publications, which reported a nondiagnostic cytology rate of approximately 6.5%.[20,21] Nondiagnostic cytology often represents a dilemma in the clinical management of patients.[22] These patients are generally referred for either repeated FNA or surgical resection for diagnostic purposes. At our institution, the surgical rate of patients who had nondiagnostic cytology was approximately 10%, which is lower than the rate reported in other series.[23,24] Coorough et al.[20] reported a surgical rate of 24% in patients with nondiagnostic cytology and detected malignancy in approximately 12% of these patients; interestingly, the occurrence of PTC was two-fold higher in patients who had nondiagnostic FNAs compared with those who had benign cytology. In our series, the ROM in this subset of patients was very high (29.8%). These findings may be justified in part by the presence of specific clinical reasons driving a surgical approach, such as compressive symptoms or suspicious ultrasound features. Another possible explanation of the high malignancy rate in nondiagnostic FNAs observed in our series is the use of only 25-gauge needles: some authors achieved better results introducing larger needles, although others were unable to find any correlation between the needle gauge and the FNA diagnostic rate.[25]

The mean age of patients who had nondiagnostic nodules that were malignant at histology was younger compared with those who had benign histology (45.6 vs. 52.6 years); moreover, men had a higher ROM compared with women. These findings could suggest more careful management of young male patients with nondiagnostic cytology.

Although still distant from an international standardization of thyroid FNA terminology, all of the most commonly used reporting systems of thyroid cytology (Bethesda, UK RCPath, JTA, and ICCRTC) propose six categories, and each category is associated with a defined ROM. Despite the substantial similarities between the

corresponding lowest and highest cytologic categories across the reporting systems, some of the most important differences regard the indeterminate categories, both in the morphologic criteria adopted and in the rate of ROM. In the revised version of the Bethesda classification,[11] the heterogeneous category of atypia of undetermined significance/follicular lesion of undetermined significance (category III) has been further subclassified according to the presence or not of nuclear atypia, architectural atypia, oncocytic features, or atypia not otherwise specified. In the Italian system, instead, the low-risk indeterminate category (TIR 3A) encompasses thyroid aspirates rich in microfollicles in the absence of nuclear atypia. This morphologic difference justifies, at least in part, the lowest ROM of TIR 3A (pooled ROM, 17%) compared with the ROM of atypia of undetermined significance/follicular lesion of undetermined significance (category III; pooled ROM, 21.5% in Western patient cohorts).[7] The follicular neoplasm/suspicious of malignancy category (category IV) of the Bethesda system encompasses thyroid aspirates that exhibit architectural atypia and mild cytologic atypia, including Hurthle cell (oncocytic) neoplasms. Similarly, in the Italian system, the high-risk indeterminate category (TIR 3B) includes both high cellularity aspirates with microfollicular/trabecular architecture and poor colloid and aspirates with some degree of nuclear atypia, insufficient to make a suspicion of PTC. Regardless of the overlapping morphologic features of TIR 3B and of category IV of the Bethesda system, the corresponding ROMs differ: 25%–40% for Bethesda category IV and 47% for TIR 3B.[7] Similarly, the JTA system reported ROMs that varied from 5% to 15% for indeterminate A-1 to 40%–60% for indeterminate A-3, showing a strong similarity to the Italian system. Conversely, the UK RCPath system reported pooled ROMs of 25% for Thy3a and 27.3% for Thy3f, which were close to the ROMs observed in the Bethesda system. The Italian and Japanese systems seem to provide a more evident increase in ROM in the indeterminate cytology subcategories compared with the Bethesda and UK RCPath systems, suggesting that the cytologic atypia described in the highest indeterminate categories of the Italian and Japanese systems is more capable of predicting malignancy than architectural atypia.

In the current study, the cumulative prevalence of indeterminate classes was 13.5%, with a large predominance of low-risk (TIR 3A) nodules with respect to high-risk (TIR 3B) indeterminate nodules (10% vs. 3.5%). This incidence is lower than that of other Italian studies, which reported that indeterminate nodules account for 20%–25% of all thyroid cytologic reports.[23,24,26] Indeterminate cytology in our series occurred less frequently even compared with other studies that used the Bethesda classification, in which the reported incidence ranged from 20% and 30%.[1] To date, the management of patients with indeterminate nodules at cytology represents a challenge for clinicians. Indeed, many nodules in this category prove to be benign at histologic evaluation; therefore, surgery is unnecessary.[26] At our institution, the choice of surgical approach was strongly related to the subcategory of indeterminate lesions: 34.3% of low-risk (TIR 3A) versus 79.8% of high-risk (TIR 3B) indeterminate nodules were referred to surgery. Moreover, the ROM was very different between the two categories of indeterminate nodules—32.5% for TIR 3A and 52.1% for TIR 3B—when considering the group of NIFTPs as nonmalignant. In the literature, the rates of malignancy for indeterminate nodules appear widely variable among different institutions, with ROMs that are usually under the cut-off of 30%–40%.[27] Our results can be considered in line with the observations for low-risk (TIR 3A) nodules. Moreover, the ROM observed for the TIR 3B class was consistent with the results of a meta-analysis performed on nodules that were diagnosed using the Italian system, in which the reported ROM for TIR 3B nodules was 52%.[24] The majority of TIR 3B nodules with malignant histology were PTCs, with a great predominance of the follicular variant of PTC. This variant of PTC is usually considered less aggressive than other PTC variants in terms of persistence and/or recurrent disease.[28,29] These findings are in line with the observation that patients who have thyroid carcinoma with presurgical indeterminate cytology usually have better outcomes than those who have suspicious or malignant cytology.[30,31] Many publications have extensively evaluated the impact of molecular testing in the management of indeterminate nodules, and the reported results appear quite variable.[32–35] The declared goal of this approach is to narrow the risk of the malignancy range to reduce the need for diagnostic surgery.[32] At our institution, molecular testing was performed in clinically selected patients, mostly in the presence of indeterminate results at cytology (12.6% of TIR 3A nodules and 12.7% of TIR 3B nodules). It has been estimated that the use of *BRAF* V600E mutation analysis alone has a specificity of approximately 99%.[36] Accordingly, in our

series, the presence of *BRAF* V600E mutation was associated with malignant histology in all cases. Nevertheless, the diagnostic value of a *BRAF* V600E mutation alone was limited because it was identified in only 0.7% of TIR 3A nodules and 4.5% of TIR 3B nodules. Conversely, *RAS*-like mutations are more frequent in the indeterminate categories, but the management of nodules carrying *RAS*-like mutations is still under debate.[37,38] In our series, among 50 *RAS*-like–positive indeterminate nodules, 32 were malignant at histology (64%), including 17 TIR 3A nodules (15 PTCs and two FTCs) and 15 TIR 3B nodules (12 PTCs and three FTCs), with a prevalence of low-risk carcinomas (follicular variant of PTC). Moreover, eight *RAS*-like–mutated TIR 3A and TIR 3B nodules (16%) were diagnosed as NIFTP. Finally, benign histology was diagnosed in 10 cases (20%), all of which were TIR 3A lesions; therefore, no mutated TIR 3B nodules were benign at histology. These findings suggest that TIR 3B indeterminate cytology, coupled with the presence of a *RAS*-like mutation, is able to predict NIFTP or low-risk malignancy with high confidence.

The main demographic findings emerging from this study on TIR 4 and TIR 5 classes are: (1) the mean age of patients who have suspicious or frankly malignant cytology is younger compared with those who have benign/indeterminate cytology; (2) the female-to-male ratio is lower in the highest cytologic classes, mostly compared with benign cytology; (3) the mean size of the nodule gradually decreases according to the increase of cytologic class; and (4) the occurrence of a single nodule is more frequently observed in the highest cytologic categories. In agreement with other authors, male sex and young age seem to confer a higher ROM.[18,39,40] In our series, the nodules classified as TIR 4 or TIR 5 at cytology had a mean size <2 cm. This finding is in line with the size distribution of thyroid cancer reported by Davies and Welch, both in their report of 2006,[41] in which the authors attributed the increase in thyroid cancer mainly to tumors that were measured ≤2 cm, and in their report of 2014,[42] in which they observed that only 33% of thyroid cancers measured >2 cm. The continuous trend of size contraction in malignant thyroid nodules probably is ascribable, at least in part, to the more frequent use of diagnostic imaging procedures.[43] In our series, the prevalence of malignancy in patients who had a solitary thyroid nodule was higher than that among those who had multinodular disease. These findings appear to be in line with some publications,[16,44] whereas other authors have reported an equal ROM between patients with solitary and multiple nodules.[45,46]

Our study has some limitations. First, some histologic reports were before the advent of NIFTP[47]; this may contribute to the high ROM of indeterminate categories, mostly TIR 3B. Second, the selection of nodules submitted to surgery cannot be straightforwardly attributed to the cytologic diagnosis. In fact, the decision to perform surgery also depends on other factors, including ultrasound characteristics of the nodule, medical judgement, and patient preferences. Herein, strictly clinical information and follow-up data have not been considered because they were beyond the aim of this study. Finally, the number of thyroid nodules submitted to molecular analysis is relatively small, and this could weaken our observations related to the utility of molecular testing.

Although the identification of aggressive variants of PTC on cytology is not emphasized in the main diagnostic guidelines, our findings demonstrate the high reliability of FNA in identifying preoperatively aggressive histotypes, as confirmed by other authors.[30] Indeed, if we exclude only a few cases of poorly differentiated carcinoma labeled as indeterminate and anaplastic carcinoma and defined as nondiagnostic on FNA, the large majority of aggressive variants of PTC (i.e., tall cell variant) has been classified as suspicious or frankly malignant. Conversely, this study highlights the finding that malignancies with follicular architecture, including FTC, the follicular variant of PTC, and NIFTP, are often diagnosed preoperatively as indeterminate and, less frequently, as benign nodules. In this regard, the detection of *RAS*-like mutations increases the ROM, but still with a limited specificity.

AUTHOR CONTRIBUTIONS

Liborio Torregrossa: Conceptualization, methodology, validation, investigation, resources, data curation, writing–initial draft, writing–review and editing, and visualization. **Anello Marcello Poma:** Conceptualization, software and formal analysis, methodology, validation, investigation, resources, data curation, writing–initial draft, writing–review and editing, and visualization. **Elisabetta Macerola:** Conceptualization, methodology, validation, investigation, resources, data curation, writing–initial draft, writing–review and editing, and visualization. **Teresa Rago:** Methodology, validation, investigation, resources, data curation, writing–review and editing, and visualization. **Paola Vignali:** Conceptualization, methodology, validation, investigation, resources, data curation, writing–review and editing, and visualization. **Rossana Romani:** Methodology, validation, investigation, resources, data curation, writing–review and editing, and visualization. **Agnese Proietto:** Methodology,

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CONFLICTS OF INTEREST

The authors made no disclosures.

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