Bethesda Classification and Cytohistological Correlation of Thyroid Nodules in a Brazilian Thyroid Disease Center

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Keywords

Thyroid nodule · Bethesda system · Thyroid cytology · Fine needle aspiration · Risk of malignancy

Abstract

Background: The Bethesda System has been used to classify thyroid cytology in 6 categories besides presenting malignancy rates and respective approaches. Reference centers have validated its use by comparing its proposed malignancy rates with those in their populations. However, to the best of our knowledge, there has been no corresponding study in Brazil. **Objectives:** To evaluate the performance of the Bethesda classification in a Brazilian thyroid reference center and correlate the results with cytohistological reports in patients referred to surgery. **Methods:** Data records from 980 fine-needle aspiration (FNA) results were retrospectively analyzed, and, in patients who underwent surgery, the results were correlated with the cytohistological findings. **Results:** 980 FNAs and 585 patients were evaluated. The inci-

dence of each cytological category was: 11% nondiagnostic (ND), 59.6% benign, 7.1% (atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS), 8.5% follicular neoplasm or suspicious for follicular neoplasm (FN/SFN), 5.1% suspicious for malignancy (SM), and 8.3% malignant. The surgery rate was 41.8% (245/585). The malignancy rate in each category was: 6% benign, 12% AUS/FLUS, 20.8% FN/SFN, 72.5% SM, and 97.3% malignant. For ND nodules, the malignancy rate was 25.7% (66.6% multifocal and papillary microcarcinomas), a higher rate than in the literature. In this category, surgery was performed in multinodular goiters presenting with another nodule >3.0 cm and/or with an FN/SFN, SM, or malignant cytological result. **Conclusion:** The Bethesda System can be applied to the Brazilian population, since the frequency and malignancy rates of each category were similar to those described by its classification. It is noteworthy that a higher risk of malignancy was observed in the ND cytological category.

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Introduction

Epidemiological studies show that thyroid nodules are palpable in approximately 5% of women and 1% of men living in iodine-sufficient areas [1–3]. Using thyroid ultrasonography (US), this prevalence increases to 19–67%, with a predominance in females, the elderly, and populations living in areas with insufficient iodine consumption [1–4]. When a nodule is diagnosed, thyroid function is then assessed; in the presence of a normal level of thyroid stimulating hormone, it is important to exclude thyroid carcinoma, which occurs in approximately 5–15% of nodules, 95% of which are well-differentiated thyroid carcinoma [1–6].

In Brazil, the current estimated incidence of thyroid cancer is 24 cases per 100,000 inhabitants, and it is the fourth-most common neoplasm found in women [1, 2, 7]. As thyroid US has become widely used, a higher number of thyroid nodules have been detected, resulting in an increased diagnosis of thyroid cancer, particularly microcarcinomas (<10 mm in diameter) [8–10].

Some sonographic features, such as hypoechogenicity, the presence of microcalcifications, irregular margins, and a taller than wide shape, are used to evaluate the risk of malignancy, but these findings alone cannot be used to distinguish between benign and malignant lesions [3, 4]. In general, a cytological analysis with fine-needle aspiration (FNA) under US guidance is recommended for nodules with intermediate or highly suspicious sonographic patterns and with the greatest dimension being at least 1 cm [1, 3].

The Bethesda System for Reporting Thyroid Cytopathology [11], published in 2007, has been widely used. It classifies the cytological results into 6 categories and presents their respective malignancy rates and approaches to treatment. These 6 categories are: nondiagnostic or unsatisfactory (ND, Bethesda I), benign (Bethesda II), atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS, Bethesda III), follicular neoplasm or suspicious for follicular neoplasm (FN/SFN, Bethesda IV), suspicious for malignancy (SM, Bethesda V), and malignant (Bethesda VI) [11–13].

Since its publication, several centers in different countries have applied the Bethesda classification system to their population, reporting a correlation between their cytological findings and malignancy rates in each category [14–21]. The majority of these studies found similar incidence rates for each category but different malignancy rates, mainly in the ND and AUS/FLUS categories [14–19]. Some questions have arisen over the proper use of the

Bethesda diagnostic categories, their associated risks of malignancy and appropriate management, leading to a recently published revision of the guidelines in 2017 [13].

Although the Bethesda classification is currently used in Brazil, there are no data related to the prevalence of each cytological category for the evaluation of thyroid nodules or the validation of whether the malignancy rates reported in the literature could be applied to the Brazilian population. The aim of this study was to evaluate the performance of the Bethesda classification in a Brazilian reference center and correlate cytological and histopathological results in patients undergoing surgery.

Materials and Methods

Patients

After approval from the Institutional Ethics Committee, we retrospectively collected data from FNA results obtained between January 2012 and December 2013 in the Thyroid Diseases Center at the Division of Endocrinology, Department of Medicine, Universidade Federal de São Paulo, SP, Brazil.

All patients were referred to the reference center when diagnosed with thyroid nodules, and they underwent thyroid palpation and a neck US. If a thyroid nodule with at least 1 cm in the greatest dimension and/or suspicious sonographic characteristics for malignancy was detected (hypoechogenicity, microcalcifications, irregular margins, and a tall shape), an FNA under US guidance was performed. The cytological results of each nodule were classified according to the Bethesda classification, and then compared with histopathological data obtained from the patients who underwent surgery.

A total of 585 patients were evaluated, including patients with >1 nodule. A total of 980 FNAs were performed, and 50 nodules were submitted to a second FNA during follow-up. Ninety percent of the patients were women, and the mean age of the patients was 51 years (range 16–83 years). The surgery rate was 41.8% (245 patients).

Neck US and Cytological Analysis

Neck US examinations were performed with a linear, multi-frequency transducer (7.5–10 MHz) integrated with color Doppler and all were performed by the same radiologist. The samples obtained by FNA were fixed and stained by panoptic techniques, and the analysis of all samples was performed by the same pathologist.

Follow-Up

Patients with nodules classified as benign were referred to clinical follow-up. Patients with nodules classified as FN/SFN, SM, or malignant underwent surgery. Patients with nodules classified as ND or AUS/FLUS underwent another FNA, with an interval of approximately 3 months in between. After the second FNA, surgery was indicated if the cytological results were reclassified as FN/SFN, SM, or malignant, or if they were reclassified in the same category (ND or AUS/FLUS) but with significant nodule growth or suspicious sonographic patterns. Surgery was also indicated if the

Fig. 1. Repeated fine-needle aspiration in AUS/FLUS category. AUS/FLUS, atypia of undetermined significance or follicular lesion of undetermined significance; FN/SFN, follicular neoplasm or suspicious for follicular neoplasm; SM, suspicious for malignancy.

nodule, in any category, presented with a large size (>3.0 cm), significant growth during follow-up, or SM characteristics on US, or if the patient had compressive symptoms.

Statistical Analysis

The incidence of each cytological category was calculated by dividing the number of FNAs in each category by the total number of FNAs performed during the study. The malignancy rates for each category were calculated by dividing the total number of malignant histological results by the total number of cases submitted to surgery.

Indices relating to the FNA procedure, such as sensitivity, specificity, positive predictive value (VPP) and negative predictive value (VPN), were calculated using the formulae of Galen and Gambino [22]. True-negative samples were considered to be nodules with both a benign cytology and histology, and true-positive samples were considered to be nodules with undetermined (FN/SFN or SM) or malignant cytology, and malignant histological results. Samples classified as ND and AUS/FLUS were excluded from the analysis due to the indication for repeated FNA instead of surgical excision. For nodules submitted to multiple FNAs, the cytological sample that was the most suspicious was considered and correlated with the histological result.

Results

According to the Bethesda classification, the cytological results in the 980 nodules were: 11% ($n = 108$) ND, 59.8% ($n = 587$) benign, 7.1% ($n = 70$) AUS/FLUS, 8.5% ($n = 84$) FN/SFN, 5.1% ($n = 50$) SM, and 8.2% ($n = 81$) malignant.

ND according to Bethesda I

Regarding the 108 nodules classified as ND, 27 were submitted to a second FNA and 20 (74%) were reclassified as: benign ($n = 14$, 70%), AUS/FLUS ($n = 4$, 20%), FN/SFN ($n = 1$, 5%), and SM ($n = 1$, 5%). The other 7 (25.9%) remained classified as ND. Six of the 27 nodules

were resected; 5 (1 ND, 3 benign, and 1 FN/SFN) had an histology report of benign, and 1 (also benign) presented with a follicular microcarcinoma.

A total of 47/108 nodules were followed up with periodic US with no changes in size or sonographic pattern. Thirty-five of 108 nodules were submitted to surgery in the presence of another nodule in the gland >3 cm (28.5%) in size, in the presence of another nodule with a suspicious cytological result (57.2%), or both (14.3%). Surgical procedures for these patients were indicated due to the presence of another nodule in the gland (rather than the ND).

The malignancy rate in nodules classified as ND was 25.7% (9/35), with 66.6% (6/9) being papillary microcarcinomas. The remaining tumors were 2 papillary carcinomas (1.3 cm in size) and 1 follicular carcinoma (1.5 cm in size). Among the malignant lesions in the ND category, 6/9 were found as multifocal tumors and 4 of them were papillary microcarcinomas.

Benign according to Bethesda II

Regarding the 587 nodules classified as benign, 12 were submitted to a second FNA and were reclassified as follows: 10 (83.3%) remained benign and 2 (16.7%) were considered as FN/SFN. Six of the 12 nodules were resected; 3 presented histologically benign results (reclassified as benign), and 3 presented as papillary carcinoma (2 reclassified as FN/SFN and 1 as benign). The median tumor size in the reaspirated group was 2.1 cm.

After the first FNA, 166/587 nodules were resected due to the size of the nodule (>3.5 cm) or the presence of another suspicious cytological nodule in the gland. The malignancy rate in this category was 6% (10/166), with a tumor size ranging from 0.4 to 6.4 cm. The remaining 409 nodules were followed up with periodic US.

Table 1. Resected nodules in each Bethesda category and correlation with cytohistological results

Cytological category	Nodules submitted to surgery		Malignancy rates	
	<i>n</i>	%	<i>n</i>	%
Nondiagnostic (<i>n</i> = 108)	35	32.4	9	25.7
Benign (<i>n</i> = 587)	166	28.2	10	6
AUS/FLUS (<i>n</i> = 70)	25	35.7	3	12
FN/SFN (<i>n</i> = 84)	77	91.6	16	20.8
SM (<i>n</i> = 50)	40	80	29	72.5
Malignant (<i>n</i> = 81)	75	92.5	73	97.3
Total FNA (<i>n</i> = 980)	418	42.6	140	33.4

AUS/FLUS according to Bethesda III

An AUS/FLUS classification was assigned to 7.1% of the FNAs performed (70/980). Eleven of 70 nodules were reaspirated and reclassified (Fig. 1). After a second FNA, 7 nodules were resected; 4 presented histologically benign results (2 benign, 1 FN/SFN, and 1 SM), 2 presented as papillary carcinoma (1 SM and 1 malignant), and 1 presented a fibromyxoid sarcoma located near the thyroid gland (reclassified as SM).

A total of 25/70 nodules were submitted to surgery due to the size of the nodule or the presence of another suspicious cytological nodule in the gland. The malignancy rate in this category was 12% (3/25) and the average tumor size was 1.3 cm (range 0.8–2 cm). The other 34 nodules were followed up with periodic US.

FN/SFN, SM, and Malignant according to Bethesda IV, V, and VI, Respectively

A total of 215 nodules classified as FN/SFN (*n* = 84), SM (*n* = 50), and malignant (*n* = 81) were referred to surgery, and data from 192 of these were included in the analysis. At the end of this study, 23 patients had not yet undergone surgery due to unforeseen circumstances, such as patient dropout and surgery refusal.

Among the 84 nodules classified as FN/SFN, 77 were resected. The malignancy rate was 20.8% (16/77) and the tumor sizes ranged from 1.3 to 6.9 cm. Among the 50 nodules classified as SM, 40 were resected. The malignancy rate in this category was 72.5% (29/40), with a predominance of papillary carcinomas. The tumor sizes ranged from 0.5 to 8.5 cm. Among the 81 nodules classified as malignant, 75 were resected. The malignancy rate in this category was 97.3% (73/75), with a predominance

of papillary carcinomas, and 2 medullary carcinomas, 1 follicular carcinoma, and 1 anaplastic carcinoma. The tumor sizes ranged from 0.4 to 6.9 cm.

The percentage of cases submitted to surgery and their respective cytohistological results are described in Table 1.

Considering the categories of FN/SFN, SM, and malignant with their respective histological results, the sensitivity, specificity, VNP and VPP were 92.1, 67.8, 93.9, and 61.4%, respectively.

Regarding the 140 (33.4%) malignant histological results, papillary carcinomas were diagnosed in 93.6% (131/140), medullary carcinoma in 2.9% (4/140), follicular carcinoma in 2.1% (3/140), anaplastic carcinoma in 0.7% (1/140), and fibromyxoid sarcoma located near the thyroid gland in 0.7% (1/140).

Discussion

Prior to the Bethesda System for Reporting Thyroid Cytopathology, there was no standard classification and no application of reproducible cytological reporting of thyroid nodule FNA results. The Bethesda System proposed the classification of 6 categories of cytological results, with the aim of standardizing the findings described by different interobservers, reporting malignancy rates, and applying a different approach in each category [11–13].

In this study, the incidence of each cytological category was in accordance with that proposed by the Bethesda classification and literature [3, 12], i.e., 11% ND (2–20%), 59.8% benign (55–74%), and 7.1% AUS/FLUS (up to 7% according to The Bethesda System and 2–18% in the literature). The incidences of FN/SFN and SM, compared with the literature, were 8.5% (2–25%) and 5.1% (1–6%), respectively [3, 12], but these frequencies are not reported by The Bethesda System [11, 12]. The incidence of malignant FNA found in this study (8.2%) was higher than that observed in the literature (2–7%), but this was to be expected, as the study was carried out in a thyroid reference center.

The Bethesda System proposes that ND nodules should be submitted to a second FNA at an appropriate interval; in many cases (50–88%) [11, 12], this second FNA allows them to be reclassified into a distinct category. In this study, 27/108 (25%) of the ND nodules were submitted to a second FNA, and 20 of these 27 (74%) were reclassified into a different category: 70% (14/20) benign (41.7 to 87.5% in the literature), 20% (4/20) AUS/FLUS, 5% (1/20)

FN/SFN, and 5% (1/20) SM. Seven of the 27 (25.9%) remained classified as ND after a second FNA, which is in accordance with the literature (1.2–47.9%) [16–19]; however, this finding is higher than that suggested by the Bethesda classification (7–10%) [11, 12]. In this study, the comparatively low percentage of ND nodules submitted to another FNA, i.e., 25% of the total, probably contributed to this result, which could turn out to be different if all of the ND nodules had been submitted to a second procedure.

Clinical follow-up is recommended for nodules classified as benign (Bethesda II). If significant growth or sonographic changes are observed, another FNA should be performed [11, 12]. In this study, 12/587 (2%) benign nodules were submitted to a second FNA; 10 (83.3%) remained benign and 2 (16.6%) were reclassified as FN/SFN (Bethesda IV). In the literature, Yang et al. [16] performed a second FNA in 11.7% benign nodules; 82.1% of these remained benign and 9.1% were reclassified.

According to The Bethesda System, AUS/FLUS nodules (Bethesda III) should be submitted to another FNA after an adequate interval, and the majority of them are reclassified into another category [11]. In this study, 50% of AUS/FLUS nodules were reclassified as benign after a second FNA, in agreement with the literature (42.7–73.1%), which allows a clinical follow-up approach instead of a surgical procedure [16, 23–25]. On the other hand, 9% of AUS/FLUS nodules submitted to a second FNA remained in the same category. This result was in agreement with what was proposed in the Bethesda classification (approx. 20%) [11, 12] and in the literature (3.8–38.5%) [16, 23–25].

In this study, the malignancy rates obtained in each cytological category were in agreement with those proposed by the original Bethesda classification, the revised Bethesda System [13], and the literature: 6% in benign nodules (0–3%, The Bethesda System; 1–10%, the literature), 12% in AUS/FLUS (5–15%, the original Bethesda classification; 10–30%, the revised Bethesda System and the literature), 20.8% in FN/SFN (15–30%, the original Bethesda classification and the literature; 25–40%, the revised Bethesda System), 72.5% in SM (60–75%, the original Bethesda classification and the literature; 50–75%, the revised Bethesda System), and 97.3% in the malignant category (97–99%, the original Bethesda classification, the revised Bethesda system, and the literature) [3, 11–19, 25].

The malignancy rate obtained in the ND category was 25.7%, with 67% of the cases being microcarcinomas. This result was in agreement with previous studies pub-

lished in the literature (10–35%) [14–17], but higher than that proposed by the original and the revised Bethesda System (1–4% and 5–10%, respectively) [11–13]. One possible reason for this finding is that the majority of ND nodules were submitted to surgery before performing a second FNA, due to the presence of other suspicious nodules in the gland or a nodule size >3 cm. If those nodules had been submitted to a second FNA, they probably would have been reclassified in the suspicious category.

In conclusion, this study found that The Bethesda System can be applied to the Brazilian population since the frequency of each category and malignancy rates were similar to those described by the system's classification. However, a higher malignancy rate was observed in the ND category. In the presence of a multinodular goiter with an ND result and another nodule >3.0 cm, or with another suspicious cytological nodule, the occurrence of multifocal tumors should be carefully evaluated.

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Disclosure Statement

R.P.M.B. and R.M.B.M. are investigators of the Fleury Group. The authors have no conflict of interest to disclose.

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