Rates of Malignancy in Cytology Indeterminate Thyroid Nodules: A Single Center Surgical Series

Lior Baraf MD[1]*, Yuval Avidor MD[2]*, Anat Bahat Dinur MD[2], Uri Yoel MD[1], Benzion Samueli MD[3], Ben-Zion Joshua MD[2], and Merav Fraenkel MD[1]

Departments of [1]Endocrinology, [2]Otolaryngology, and [3]Pathology, Soroka University Medical Center, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel

ABSTRACT **Background:** Due to the high variability in malignancy rate among cytologically indeterminate thyroid nodules (Bethesda categories III–V), the American Thyroid Association recommends that each center define its own categorical cancer risk.

Objectives: To assess cancer risk in patients with cytologically indeterminate thyroid nodules who were operated at our center.

Methods: In a retrospective study, we analyzed the pathology results of all the patients whose fine needle aspiration results showed Bethesda III–V cytology and who subsequently underwent total thyroidectomy or lobectomy from December 2013 to September 2017.

Results: We analyzed 56 patients with indeterminate cytology on fine needle aspiration. Twenty-nine (52%) were defined as Bethesda III, 19 (34%) Bethesda IV, and 8 (14%) Bethesda V category. Malignancy rates were 38%, 58%, and 100% for Bethesda categories III, IV, and V, respectively. Most malignancies in Bethesda categories III and IV were follicular in origin (follicular thyroid carcinoma and follicular type papillary thyroid carcinoma), while 100% of the patients with Bethesda category V were diagnosed with classical papillary thyroid carcinoma. No correlation was found between sonographic and cytological criteria of nodules with Bethesda categories III and IV and rates of malignancy.

Conclusions: We found higher than expected rates of malignancy in indeterminate cytology. This finding reinforces the guidelines of the American Thyroid Association to establish local malignancy rates for thyroid nodules with indetermined cytology.

IMAJ 2023; 25: 147–151

KEY WORDS: Bethesda classification, cytology, fine needle aspiration, thyroid cancer, ultrasound

*These authors contributed equally to this study

The detection of thyroid nodules in clinical practice has increased [1]. Nonetheless, most nodules are benign in nature [2]. The 2007 National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference provided consensus recommendations known as the Bethesda System for Reporting Thyroid Cytopathology [3,4]. The Bethesda system recognizes six diagnostic categories and provides an estimation of cancer risk within each category based on literature review and expert opinion. Bethesda categories I, II, and VI are considered non-diagnostic, benign, and malignant, respectively. For categories III (atypia of undetermined significance or follicular lesion of undetermined significance), IV (follicular neoplasm or suspicious for a follicular neoplasm), and V (suspicious for malignancy), the estimated risks for malignancy are 5–15%, 15–30%, and 60–75%, respectively, and are thus considered as intermediate risk.

A major problem in interpreting cytological findings in the Bethesda era is the high variability among pathologists and institutions in the rates of malignancy in Bethesda categories III, IV, and V [5]. Difficulties in determining the actual risk of malignancy may arise from surgical bias (in Bethesda III) and from the subjective component of cytological examinations, as evident by interobserver discrepancy [6].

Assessments of malignancy rates for these categories may have important implications on patient management (e.g., surgery vs. follow-up in Bethesda categories III and IV) and on the extent of surgery (total vs. hemi-thyroidectomy in Bethesda category V). Consequently, the 2015 American Thyroid Association guidelines recommend that each center defines its own categorical cancer risk of indeterminate cytology [5].

To date, most studies reporting categorical risk of malignancy were surgical series, and thus not representative of the entire population of patients with cytologically indeterminate nodules [7]. Others, like the study by Godoi Cavalheiro et al. [8], in which all Bethesda III (N=478) and IV (N=137) nodules were operated, reported a lower rate (17%) of malignancy for Bethesda IV but surprisingly, not for Bethesda III (16%). Attempts have been made to refine the risk of malignancy of nodules with indeterminate cytology, according to cytological features and molecular testing [9-11]. These studies found that nuclear atypia confers a significantly higher risk for malignancy than does architectural atypia.

The aim of this study was to assess cancer risk in cytologically indeterminate thyroid nodules in patients who had fine needle aspiration cytology performed by the Department of Pathology and were operated at the Department of Otolaryngology at our center.

PATIENTS AND METHODS

Data were collected retrospectively of patients who underwent total thyroidectomy or lobectomy at the Department of Otolaryngology at our center between December 2013 and September 2017. Included in the study were all patients with indeterminate cytology results (Bethesda category III–V) on fine needle aspiration performed at our institution.

The data collected for analysis included demographic characteristics, neck and thyroid ultrasonography features prior to surgery, cytology analysis, and pathology from thyroid surgery. The demographic characteristics included age, sex, ethnicity, family history of thyroid cancer, and previous exposure to ionizing radiation. Neck and thyroid ultrasonography was performed at several radiology centers and by a number of ultrasonography specialists. Ultrasound images were not reviewed; rather, reports were used for data extraction. Data extracted included findings from both lobes of the thyroid and the isthmus: size, single vs. multiple nodules, bilateral vs. unilateral nodules, and nodule description (e.g., size, echogenicity, borders, calcifications, vascularity, dimensions). Cytology was analyzed by several pathologists from our institution. Three reports that did not include a Bethesda classification were not included in the analysis. Cytology reports of fine needle aspiration were reviewed, and data were extracted. These included the number and type of cytological criteria [4] that were mentioned for the diagnosis of the three Bethesda groups analyzed (III–V). Pathologists from our institution assessed pathology.

The histopathological results from thyroidectomy were reviewed. Only the findings that were related to the nodules that were diagnosed with intermediate cytology by fine needle aspiration were analyzed. Incidental and unrelated findings were excluded. Surgical pathology reports were categorized as benign or malignant (papillary thyroid cancer [PTC]/follicular thyroid cancer [FTC]). The PTC group was subcategorized to classical and follicular variants.

Clinical, cytological, and ultrasonographic features were compared for each Bethesda cytology group III–V, between those with benign and malignant pathologies. Neither repeated FNA nor molecular studies were performed in our cohort.

The results are presented as mean ± standard deviation for continuous and ordinal variables (age, maximal diameter, the number of suspicious features in ultrasound, and the number of cytological criteria) and as percentages for categorical data (patient's sex). Patient age, the number of suspicious features in ultrasound, and the number of cytological criteria in Bethesda III and IV were compared using the Mann-Whitney test. The proportion of females was compared using the chi-square test or Fisher's exact test. A *P*-value of < 0.05 was considered statistically significant. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 25 (SPSS, IBM Corp, Armonk, NY, USA).

The study protocol was approved by the institutional review board of Soroka Medical Center. Informed consent was waived due to the retrospective study design.

RESULTS

During the study period, 253 patients underwent total thyroidectomy or lobectomy in the Department of Otolaryngology at our center. The analysis included the 56 patients whose cytology results of fine needle aspiration were concluded as indeterminate. The mean age was 50 years, 74% were female. Twenty-nine (52%) were defined cytologically as Bethesda III, 19 (34%) Bethesda IV, and 8 (14%) Bethesda V category [Table 1].

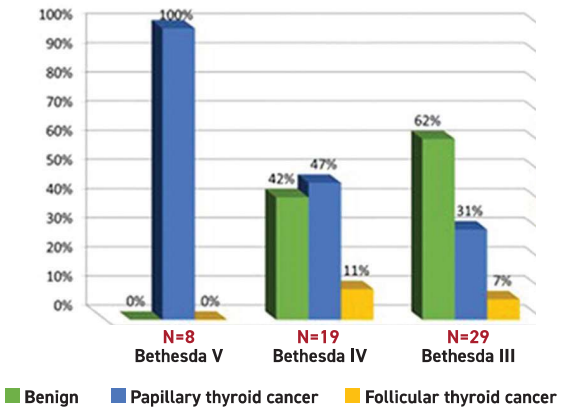
The malignancy rate in Bethesda III category was 38% [Figure 1]. Most were classified as follicular (seven as follicular type PTC, two as FTC) and only two as classical PTC. Among the patients with Bethesda category III cytology, the mean age of those with a malignant pathology was significantly younger than of those with a benign pathology [Table 2]. Sex, maximal diameter in ultrasound, the number of suspicious features on ul-

Table 1. Baseline characteristics according to Bethesda category

Bethesda category	III, n=29	IV, n=19	V, n=8	*P*-value
Benign pathology, n (%)	18 (62)	8 (42)	0	
Age in years, mean ± SD	51.82 ± 15.0	48.63 ± 15.1	49.12 ± 9.3	0.873
Sex, female, n (%)	17 (58%)	17 (89%)	6 (75%)	0.021
Max diameter in ultrasound (mm), mean ± SD	39.75 ± 20.4	31.05 ± 18.5	22.25 ± 7.7	0.024
Number of suspicious features in ultrasound, median [Q1–Q3]	1 (1–2)	2 (1–2.5)	2 (1–2.25)	0.17

Q = quarter, SD = standard deviation

Figure 1. Malignancy rate by Bethesda category



trasound, and the number of cytological criteria for diagnosis did not differ statistically between those with benign and malignant pathologies.

The malignancy rate in Bethesda IV category was 58% [Figure 1]. As with Bethesda III category, most were classified as follicular (five as follicular type PTC and two as FTC) and only four as classical PTC. Among the patients with Bethesda IV category cytology, those with a malignant pathology had significantly fewer cytological criteria for diagnosis than did those with a benign pathology result [Table 2]. Age, sex, maximal diameter in ultrasound, and the number of suspicious features on ultrasound were not statistically different between the benign and malignant pathology groups.

The malignancy rate in Bethesda V category was 100% [Figure 1]. All were classified as classical PTC. The number of suspicious features on ultrasound [Table 1] for Bethesda V was higher than for Bethesda categories III and IV, but this difference did not reach statistical significance. The diameter of Bethesda V nodules was significantly smaller than of Bethesda III and IV nodules.

DISCUSSION

In this single center study of patients with thyroid nodules of indeterminate cytology, the rates of malignancy were 38%, 58%, and 100% for Bethesda III, IV, and V, respectively. These rates are high compared to those reported in the literature.

Like most reports of malignancy rates of nodules with indeterminate cytology according to fine needle aspiration results, our cohort was a surgical series. Such studies are biased by factors involved in the decision to operate. These factors include personal risk for malignancy, growing nodules on serial sonography, and neck lymphadenopathy. These characteristics may explain the relatively high rate of malignancy of Bethesda III category nodules (38%), although this rate was within the reported range of malignancy for this category.

For Bethesda IV and V, we found higher rates (58% and 100%, respectively) for malignancy than those reported in the literature. Possible explanations are underestimation or excessive caution in interpretation of cytological findings by the reporting pathologist.

In recent years, a few single center studies [12-15] and one multicenter study [16] from Israel reported rates of malignancy in thyroid nodules with indetermined cytology. Rates of malignancy according to cytology classification from these studies in addition to the current study are summarized in Table 3. Rates of malignancy in Bethesda III–V in all five reports from Israel showed higher rates of malignancy than those reported by other studies. This finding suggests the possible involvement of environmental or genetic factors in the development of malignancy and a tendency by local pathologists to underestimate malignancy risk in thyroid cytology.

All the nodules that were classified as Bethesda V on cytology were classified as classical PTC on final postsurgical pathology. In contrast, in Bethesda III and IV, most nodules were classified as follicular or follicular variant PTC and only a minority as classical PTC (2/11 in Bethesda III and 4/11 in Bethesda IV). These results can be explained by the inclusion of papillary structures in the diagnostic criteria of Bethesda V only in the classical variant PTC and not in the follicular type.

Accumulating evidence [17] shows that among the cytological characteristics of Bethesda III and IV categories, nuclear atypia represents a higher risk of malignancy. Due to the retrospective nature of our study, we relied on the existing reports of the cytologists in which the detailed criteria were not uncommonly under reported. Therefore, we could not draw conclusions as to the association of the type of cytological criteria with malignancy rates. Alternatively, we compared the total number of Bethesda criteria between the malignant and benign nodules that were categorized as Bethesda III and IV categories. We did not find a difference in the number of criteria in the Bethesda III category. Unexpectedly, we found significantly fewer criteria in the malignant group in Bethesda IV. The small sample size and the examination by a number of cytologists could explain these results.

Table 2. Benign versus malignant cytology in Bethesda III, IV category

	Bethesda III			Bethesda IV		
	Benign, n=18	**Malignant, n=11**	***P*-value**	**Benign, n=18**	**Malignant, n=11**	***P*-value**
Age in years, mean ± SD	57.00 ± 15.1	43.36 ± 20.8	**0.024**	45.60 ± 11.2	50.80 ± 17.6	0.508
Sex, female, n (%)	11 (61)	6 (55)	0.728	7 (87)	10 (90)	0.811
Maximum diameter in ultrasound (mm), mean ± SD	44.83 ± 20.8	30.60 ± 17.0	0.058	31.5 ± 16.8	30.6 ± 20.9	0.923
Number of suspicious features in ultrasound, median [Q1–Q3]	1 (1–2)	1 (1–2)	0.579	1.5 (0.75–2)	2 (1–3)	0.370
Number of cytological criteria, median [Q1–Q3]	1 (1–2)	2 (1–3)	0.738	3 (2.75–3.5)	2 (0–3)	**0.024**

Q = quarter, SD = standard deviation

Table 3. Malignancy rate in indetermined cytology from single center studies [12-15] and from a multicenter [16] study in Israel

Study reference	Total Number of patients operated	Patients in each category (III, IV, V)	Malignancy rate		
			Bethesda III	Bethesda IV	Bethesda V
Cohen et al. [12]	46	no data	32%	no data	
Ronen et al. [13]	28	11, 6, 11	29%	50%	83%
Hirsch et al. [14]	123	66, 57, ND	45%	63%	no data
Al-Kurd et al. [15]	176	14, 162, ND	36%	64%	no data
Madgar et al. [16]	310	70, 133, 107	41%	41%	87%
Current	56	29, 19, 8	38%	58%	100%
Average			38%	55%	90%

We found that younger age was a significant risk factor for malignancy among patients classified with Bethesda III but not in the Bethesda IV category. Similarly, Todorovic et al. [18] reported significantly higher rates (36.8 vs. 7.4%) of malignancy in younger (< 55 years of age) than in older (≥ 55 years of age) patients with Bethesda III cytology [13] but did not find this association among those with Bethesda IV category nodules. These results may be due to an increased risk factor for thyroid cancer among younger people [19]. Alternatively, Bethesda III cytology may represent a specific risk for cancer among younger individuals. While more research is needed, younger age should be considered in assessing the rate of malignancy in the Bethesda III category.

Several studies have shown no association between nodule size and risk for malignancy [20-24]. However, other studies reported that larger nodules confer a higher risk for malignancy. We found that the maximal diameter of the nodule by ultrasound decreased in the progression from Bethesda III to IV to V, while the number of suspicious features on ultrasound increased but without statistical significance. This observation is not surprising due to the retrospective design of our study and its reflection of real-life clinical experience, which is characterized by a tendency to aspirate suspicious nodules irrespective of their size.

The present study has several limitations. First, the final cohort for analysis was relatively small. As a retrospective surgical series, the results may overestimate the real malignancy rate in the Bethesda III category. In addition, cytologists analyzed the cytology. The cytology report was not standardized and therefore not homogeneous. This finding limits drawing conclusions regarding the number and type of cytological criteria in each cytological subtype and the ability to compare these to the final post-surgical pathology diagnosis. Another limitation is that the sonographic examinations were conducted at different facilities and by different sonographers. This aspect also limits drawing conclusions regarding sonographic features that may increase the risk of malignancy in thyroid nodules with indeterminate cytology. For more reliable evaluations in future studies, more uniformity is required, both in the cytology and ultrasound reports. Last, the criteria of noninvasive follicular thyroid neoplasm with papillary like nuclear features, which represents a relatively benign condition, were published and included in the revised Bethesda reporting system after the end of our study [25]. Therefore, a small subset of our patients who were diagnosed with follicular variant PTC could represent this condition.

A strength of our study is that it was conducted at a large, single center; thus, the patients referred to surgery represented a rather homogenous group. In addition, the real-life nature of our study encourages other centers to collect similar data for their institutions.

CONCLUSIONS

In our retrospective, single center study we found higher than expected rates of malignancy in thyroid nodules with indeterminate cytology on FNA. The 100% malignancy rate for Bethesda V category in our institute makes this Bethesda category highly suspicious. Our findings reinforce the ATA guidelines to establish local malignancy rates for thyroid nodules with indetermined cytology. Sonographic and cytological criteria within Bethesda categories III and IV did not facilitate differentiating between those with benign and malignant pathologies, and more standardized prospective studies are needed.

Correspondence

Dr. L. Baraf
Dept. of Endocrinology, Soroka University Medical Center, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva 84101, Israel
Phone: (972-8) 640-3270
Fax: (972-8) 676-8711
Email: lior.baraf@clalit.org.il

References

1. Li N, Du X, Reitzel L, Xu L, Sturgis E. Impact of enhanced detection on the increase in thyroid cancer incidence in the United States: review of incidence trends by socioeconomic status within the surveillance, epidemiology, and end results registry, 1980–2008. *Thyroid* 2013; 23: 103-10.
2. Filetti S, Durante C, Torlontano M. Nonsurgical approaches to the management of thyroid nodules. *Nat Clin Pract Endocrinol Metab* 2006; 2: 384-94.

3. Baloch Z, LiVolsi V, Asa S, et al. Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. *Diagn Cytopathol* 2008; 36: 425-37.
4. Cibas E, Ali S. The Bethesda System for Reporting Thyroid Cytopathology. *J Am Soc Cytopathol* 2009; 132: 658-65.
5. Haugen BR. The 2015 American thyroid association evidence-based guidelines for management of patients with thyroid nodules and differentiated thyroid cancer: key recommendations. Practical management of thyroid cancer: a multidisciplinary approach. 2018: 17-20.
6. Olson M, Boonyaarunnate T, Aragon Han P, Umbricht C, Ali S, Zeiger M. A Tertiary center's experience with second review of 3885 thyroid cytopathology specimens. *J Clin Endocrinol Metab* 2013; 98: 1450-7.
7. Bongiovanni M, Spitale A, Faquin W, Mazzucchelli L, Baloch Z. The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. *Acta Cytologica* 2012; 56 (4): 333-9.
8. Godoi Cavalheiro B, Kober Nogueira Leite A, Luongo de Matos L, et al. Malignancy rates in thyroid nodules classified as Bethesda Categories III and IV: retrospective data from a tertiary center. *Int J Endocrinol Metab* 2017; 16 (1): e12871.
9. Rosario P, Calsolari M. Importance of cytological subclassification of thyroid nodules with Bethesda Category III cytology (AUS/FLUS) into architectural atypia only and nuclear atypia: a prospective study. *Diagn Cytopathol* 2017; 45: 604-7.
10. Rossi M, Lupo S, Rossi R, et al. Proposal for a novel management of indeterminate thyroid nodules on the basis of cytopathological subclasses. *Endocrine* 2016; 57: 98-107.
11. Valderrabano P, Khazai L, Thompson Z, et al. Cancer risk stratification of indeterminate thyroid nodules: a cytological approach. *Thyroid* 2017; 27: 1277-84.
12. Cohen O, Tzelnick S, Lahav Y, Schindel D, Halperin D, Yehuda M. Selection of atypia/follicular lesion of unknown significance patients for surgery versus active surveillance, without using genetic testing: a single institute experience, prospective analysis, and recommendations. *Thyroid* 2017; 27: 928-35.
13. Ronen O, Cohen H, Abu M. Review of a single institution's fine needle aspiration results for thyroid nodules: Initial observations and lessons for the future. *Cytopathology* 2019; 30: 468-74.
14. Hirsch D, Robenshtok E, Bachar G, Braslavsky D, Benbassat, C. The implementation of the Bethesda System for Reporting Thyroid Cytopathology improves malignancy detection despite lower rate of thyroidectomy in indeterminate nodules. *World J Surg* 2015; 39: 1959-65.
15. Al-Kurd A, Maree A, Mizrahi I, et al. An institutional analysis of malignancy rate in Bethesda III and IV nodules of the thyroid. *Am J Otolaryngol Head Neck Surg* 2019; 2 (1): 1034.
16. Madgar O, Avior G, Shochat I, et al. Thyroid malignancy rates according to the Bethesda reporting system in Israel - a multicenter study. *Eur J Surg Oncol* 2021; 47 (6): 1370-5.
17. Lim J, Nga M, Chan D, Tan W, Parameswaran R, Ngiam K. Subclassification of Bethesda Atypical and Follicular Neoplasm Categories according to nuclear and architectural atypia improves discrimination of thyroid malignancy risk. *Thyroid* 2018; 28: 511-21.
18. Todorovic E, Sheffield BS, Kalloger S, Walker B, Wiseman SM. Increased cancer risk in younger patients with thyroid nodules diagnosed as atypia of undetermined significance. *Cureus* 2018; 10 (3): e2348.Rago T, Fiore E, Scutari M, et al. Male sex, single nodularity, and young age are associated with the risk of finding a papillary thyroid cancer on fine-needle aspiration cytology in a large series of patients with nodular thyroid disease. *Eur J Endocrinol* 2010; 162: 763-70.
19. Hong MJ, Na DG, Baek JH, Sung JY, Kim JH. Impact of nodule size on malignancy risk differs according to the ultrasonography pattern of thyroid nodules. *Korean J Radiol* 2018; 19 (3): 534-41.
20. Cavallo A, Johnson D, White M, et al. Thyroid nodule size at ultrasound as a predictor of malignancy and final pathologic size. *Thyroid* 2017; 27: 641-50.
21. Magister M, Chaikhoutdinov I, Schaefer E, Williams N, Saunders B, Goldenberg D. Association of thyroid nodule size and Bethesda Class with rate of malignant disease. *JAMA Otolaryngol Head Neck Surg* 2015; 141: 1089.
22. Albuja-Cruz M, Goldfarb M, Gondek S, Allan B, Lew J. Reliability of fine-needle aspiration for thyroid nodules greater than or equal to 4 cm. *J Surg Res* 2013; 181: 6-10.
23. McHenry C, Huh E, Machekano R. Is nodule size an independent predictor of thyroid malignancy? *Surgery* 2008; 144: 1062-9.
24. Cibas E, Ali S. The 2017 Bethesda System for Reporting Thyroid Cytopathology. *Thyroid* 2017; 27: 1341-6.

Capsule

Editing away heart disease

Ischemia-reperfusion injury, tissue damage that occurs after oxygen deprivation, can be observed after a variety of insults, including common ones such as heart attack or stroke. A key protein that plays a role in this damage is calcium calmodulin-dependent protein kinase IIδ (CaMKIIδ). **Lebek** and associates found that targeting CaMKIIδ using CRISPR-Cas9 gene editing was a viable intervention to protect the heart tissue from ischemia-reperfusion damage in mouse models. Injecting gene-editing reagents soon after ischemia exposure was sufficient for the mice to recover from severe heart damage, suggesting that it may not be too late to intervene after a heart attack happens.

Sciences 2023; 379: 179
Eitan Israeli

Capsule

Microbiota and tau-mediated disease

The accumulation of certain forms of the tau protein in the brain is linked to loss of nerve cells, inflammation, and cognitive decline in Alzheimer's disease and several other neurodegenerative diseases. Apolipoprotein-E (APOE), the strongest genetic risk factor for Alzheimer's disease, regulates brain inflammation and tau-mediated brain damage; however, the gut microbiota also regulates brain inflammation. In a mouse model of tau-mediated brain injury, **Seo** et al. found that manipulation of the gut microbiota resulted in a strong reduction of inflammation, tau pathology, and brain damage in a sex- and APOE-dependent manner.

Science 2023; 379: 155
Eitan Israeli