

# The 2023 Bethesda System Predicts Aggressive Features and Histologic Subtype in Thyroid Cancer

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## Research Article

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# Abstract

**Background:** The Bethesda System for Reporting Thyroid Cytopathology (TBS) is the standard framework for interpreting fine-needle aspiration (FNA) specimens, which includes cytologic definitions, diagnostic criteria, and clinical management guideline for each category. Although primarily designed to estimate malignancy risk, whether TBS categories predict histologic subtype and tumor aggressiveness also remains unclear. Our objective was to determine the histologic type and extent of malignancy in thyroid FNAs to expand the utility of the TBS by providing more prognostic information for clinical practice.

**Methods:** A retrospective study of all thyroid FNA cases between January 2016 and February 2025 was conducted at Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University. Clinicopathologic characteristics, ultrasonographic features, cytologic diagnoses, molecular testing results, and final histologic outcomes were collected. FNA results were classified according to TBS and correlated with ultrasound features, molecular alterations, histologic subtypes, and aggressive pathologic characteristics.

**Results:** A total of 8595 patients underwent ultrasound-guided FNA, and

TBS diagnostic categories were as follows: nondiagnostic (TBS I) in 3.5% cases, benign (TBS II) 60.2%, AUS (TBS III) 8.3%, FN (TBS IV) 3.7%, SM (TBS V) 1.9%, and malignant (TBS VI) 22.4%. Final histology demonstrated stepwise increases in malignancy rates from TBS III (39.7%) and TBS IV (47.6%) to TBS V (78.0%) and TBS VI (99.2%). Higher Bethesda categories were significantly associated with malignant ultrasound features, aggressive histologic subtypes, and adverse pathologic characteristics, including extrathyroidal extension, multifocality, lymphovascular invasion, and lymph node metastasis ( $p < 0.05$ ). Moreover, the classic variant of PTC was identified in 70.9% with malignant FNA cytology compared with 25.0% with AUS, 14.4% with FN, and 10.9% with SM cytology ( $p < 0.0001$ ). Molecular profiling revealed increasing frequencies of BRAF V600E mutations across higher Bethesda categories, while high-risk alterations such as TERT, TP53, and PIK3CA mutations were predominantly observed in TBS V.

**Conclusion:** Beyond malignancy risk estimation, the 2023 Bethesda System provides important prognostic information regarding histologic subtype, molecular alterations, and tumor aggressiveness. These findings expand the clinical utility of TBS and support its role as an integrated diagnostic and prognostic tool in thyroid cancer management.

## Introduction

Fine-needle aspiration (FNA) cytology remains the cornerstone for the evaluation and management of thyroid nodules. The Bethesda System for Reporting Thyroid Cytopathology (TBS) was developed to standardize cytologic interpretation, estimate the risk of malignancy, and provide management recommendations for each diagnostic category(1–3). Since its initial introduction, TBS has been widely adopted worldwide and has undergone periodic updates to incorporate advances in diagnostic criteria, imaging techniques, and molecular testing.

The 2023 edition of the Bethesda System further refines cytologic definitions and integrates contemporary insights into molecular pathology and clinical management(3, 4). While the primary purpose of TBS is malignancy risk stratification, emerging evidence suggests that cytologic categories may also reflect underlying tumor biology, including histologic subtype, molecular profile, and clinical aggressiveness. Such prognostic information could be particularly valuable for individualized preoperative decision-making(5, 6).

However, most studies evaluating the prognostic implications of TBS categories have been conducted in Western populations(5, 7), and data from large Chinese cohorts remain limited. Moreover, whether the updated 2023 Bethesda System provides additional prognostic value beyond malignancy risk has not been comprehensively explored. In this study, we analyzed a large retrospective cohort from a high-volume tertiary center in China to investigate the associations between Bethesda categories and histologic subtype, molecular alterations, and aggressive pathologic features of thyroid cancer.

## Materials and Methods

All patients who underwent ultrasound-guided FNA for thyroid nodules between January 2016 and February 2025 at Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University were retrospectively reviewed. **Every participant agreed to participate in this study and signed a consent form.** All tissue samples were obtained with informed consent, **and the study was conducted in accordance with the Declaration of Helsinki and was approved by the Internal Review and the Ethics Board of the Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University.** Clinical, sonographic, cytologic, molecular, and histopathologic data were collected. Patients with insufficient follow-up data were excluded.

Thyroid ultrasound examinations were performed by experienced sonographers specializing in thyroid imaging. Sonographic characteristics, including nodule composition, margins, calcifications, echogenicity, and shape, were prospectively documented. Nodules were classified according to the Thyroid Imaging Reporting and Data System (TI-RADS) adopted at our institution(8, 9).

FNAs were performed by dedicated surgeons using 23- or 25-gauge needles under ultrasound guidance, typically with one to three passes, and cytologic specimens were evaluated by cytopathologists in our institution. All cytological results were classified according to the Bethesda System for Reporting Thyroid Cytopathology (TBS), with corresponding estimated risks of malignancy(3).

Molecular testing was performed for nodules classified as TBS III, IV, and V using a next-generation sequencing panel targeting 88 genes associated with thyroid tumorigenesis. Histopathologic evaluation included tumor size, histologic subtype, extrathyroidal extension, multifocality, lymph vascular invasion, and lymph node metastasis. Tumors were staged according to the American Joint Committee on Cancer (AJCC) criteria and categorized as low-, intermediate-, or high-risk disease(10).

## Statistical analysis

Statistical analyses were performed with GraphPad Prism 10.0. The chi-square test or Fisher's exact test was performed for analysis of categorical variables. Quantitative data are shown as mean±standard deviation (SD), compared using one-way analysis of variance, whereas numbers and percentage are provided for qualitative data. All tests were two-tailed, and *P*-values < 0.05 were considered statistically significant.

## Results

A total of 8595 patients underwent ultrasound-guided FNA during the study period. Data on patient demographics, sonographic features, cytology diagnosis, and final histological diagnosis were collected. Nodule cytology details based on the Bethesda categories are shown in Fig. 1A. The TBS classification show TBS I, 3.5%; TBS II, 60.2%; TBS III, 8.3%; TBS IV, 3.7%; TBS V, 1.9%; and TBS VI, 22.4%. 2960 underwent thyroid surgery and were included in the histopathologic analysis. Final histology demonstrated malignancy rates of 39.7% for TBS III, 47.6% for TBS IV, 78.0% for TBS V, and 99.2% for TBS VI (Fig. 1B), confirming a stepwise increase in malignancy risk across Bethesda categories.

Among surgical patients, most nodules were solid (91.1%), hypoechoic or very hypoechoic (78.1%), exhibited calcifications (87.0%), and demonstrated a taller-than-wide shape (71.8%). ACR TI-RADS categories 4–5 accounted for 95.4% of cases. Higher Bethesda categories were significantly associated with malignant ultrasound features, with progressive increases observed from TBS III to VI (**Table 1**).

Higher Bethesda categories were strongly associated with aggressive histologic subtypes (**Table 2**). 81.9% tall-cell variant, and 87.1% other higher-risk variants were classified as TBS VI. The classic variant of PTC was identified in 70.9% with malignant FNA cytology compared with 10.9% with SM and 14.4% with FN cytology (*p* < 0.001). In FTC, 60.7% widely invasive type was documented in malignant FNA cytology, but only 22.8% minimally invasive type were classified as TBS VI. Similarly, 84.8% poorly differentiated carcinoma, 84.8% medullary thyroid carcinoma, 73.7% anaplastic thyroid carcinoma and 89.5% metastatic non-thyroidal malignancies were also classified as malignant cytology.

86.8% thyroid cancers with central lymph node metastasis were classified as Malignant on FNA cytology, compared with 5.9% with AUS, 4.2% with FN, and 3.1% with SM cytology (*p* = 0.0002). Importantly, TBS progressing from AUS, to FN, to SM, to Malignant increasingly predicted the risk of extrathyroidal extension (*p* = 0.0004), multifocal (*p* = 0.0016), lymph vascular invasion (*p* < 0.0001), and lateral lymph node metastasis (*p* = 0.0144). In our cohort, high-risk malignancy was identified in 82.2% of cases when cytology was Malignant, but only in 4.8%, 1.7% and 11.3% when cytology was AUS, FN, or SM respectively (*p* < 0.0001). 89.4% cases were categorized as Malignant in intermediate-risk cancers, compared with 4.2% as AUS, 3.5% as FN, and 2.9% as SM cytology (*p* < 0.0001). 54.1% with AUS and FN cytology were categorized as lower-risk cancers. This compared with only 41.1% cases in Malignant cytologic group (*p* < 0.0001) (**Table 3**). Taken together, our data indicate that TBS may inform more than just the thyroid malignant risk, and may also provide clinical practice decision of the malignant types and measures of disease aggressiveness.

We also used the next-generation sequencing assay to detect 88 genes related to thyroid tumors (Fig. 2). In PTC, the mutation rate of BRAF in TBS classification III, IV, and V was 55.9%, 76.3%, and 93.8%, respectively. Noteworthy, TBS progressing from AUS, to FN, to SM increasingly predicted more BRAF mutation ( $p = 0.0005$ ). HRAS, NRAS and KRAS mutations were detected in TBS III, IV and V cases. However, in TBS III and IV, NIFTIP was often accompanied by RAS mutation, without BRAF mutation. Some TBS V cases, RAS mutation coexisted with BRAF mutation or TERT promoter mutation. PIK3CA and TERT mutations often occurred in TBS V cases and often coexisted with other gene mutations. TP53 mutation was only detected in TBS V tumors with higher-risk malignancy. RET proto-oncogene fusion and mutation were also detected. Collectively, these data showed that some genes, representing stronger invasiveness, appeared more frequently in TBS V cytology, such as TP53, PIK3CA and TERT.

## Discussion

The Bethesda System for Reporting Thyroid Cytopathology (TBS) provides a diagnostic framework which is well associated with thyroid nodule management, and plays an important role in stratifying the risk of malignancy for thyroid nodules(3, 11–13). Moreover, the 2023 edition updated current diagnostic criteria, including the latest information on molecular testing, imaging findings, and clinical management. This edition provided a more unified approach to diagnosing and reporting thyroid fine-needle aspiration (FNA) biopsies, streamlining the reporting structure by establishing a single name for each of the six major categories(14, 15). In our TBS diagnostic categories, there were nondiagnostic in 3.5% cases, benign in 60.2%, AUS in 8.3%, FN in 3.7%, SM in 1.9%, and malignant in 22.4%. A recent prospective assessment reported similar frequencies and demonstrated high sensitivity and specificity for FNA when analyzed under the 2023 criteria(16). In surgical patients, most were classified as ACR-TIRADS level 4–5. AUS, FN, SM, and malignant cytology were progressively associated with an increasing risk of histologic subtype. Our data also confirmed that the progressive risk of higher-risk disease, extrathyroidal extension, multifocal, lymph vascular invasion and lymph node metastasis progresses from AUS to FN to SM to Malignant. In addition, the mutation rate of BRAF was varied in different TBS classification cases. Some genes, representing stronger invasiveness, appeared more frequently in TBS V. In this large retrospective cohort from a high-volume tertiary center in China, we demonstrated that the 2023 Bethesda System not only accurately predicts malignancy risk but also correlates strongly with histologic subtype, molecular alterations, and aggressive pathological features of thyroid cancer.

Ultrasound is a reliable method of diagnosis for selected patients to have an ultrasound-guided fine-needle aspiration(17, 18). As cytologic diagnosis progressed from AUS to malignant, nodules increasingly exhibited solid composition, microcalcifications, hypoechogenicity, taller-than-wide shape, and higher ACR-TIRADS categories. These findings reinforce the complementary role of ultrasound and cytology in thyroid nodule evaluation and support a multimodal risk stratification approach.

Molecular testing has emerged as an important adjunct in the evaluation of indeterminate thyroid nodules(3, 6, 19, 20). Consistent with previous studies, molecular profiling revealed distinct mutation patterns(21–23). BRAF V600E mutation frequency increased significantly from TBS III to V, reflecting a

transition toward more aggressive tumor biology. In contrast, RAS mutations were more prevalent in TBS III and IV, often associated with NIFTP or follicular-patterned tumors. Importantly, high-risk molecular alterations such as TERT promoter, TP53, and PIK3CA mutations were predominantly observed in TBS V nodules and frequently coexisted with other driver mutations. Molecular testing, as a prognostic tool, may change the clinical approach and avoid overtreatment of thyroid cancer(6, 24, 25). Our findings provide a molecular basis for the observed association between higher Bethesda categories and aggressive clinical behavior, further supporting the prognostic value of cytologic classification.

Consistent with prior studies, our data confirmed a stepwise increase in malignancy rates from indeterminate to definitive malignant cytology(5, 15). The observed malignancy rates of 39.7% for TBS III, 47.6% for TBS IV, 78.0% for TBS V, and 99.2% for TBS VI are comparable to or slightly higher than previous reported cohorts(16, 26). This discrepancy may be explained by selection bias in surgical cases, referral patterns in tertiary centers, and regional differences in clinical practice. Nevertheless, the progressive increase in malignancy risk supports the robustness and reproducibility of the Bethesda System across diverse populations. Importantly, our findings extend beyond malignancy prediction by demonstrating that increasing Bethesda categories are associated with increasing degrees of tumor aggressiveness. This observation supports the notion that cytologic atypia reflects underlying tumor biology rather than merely diagnostic uncertainty.

A key strength of our study is the detailed correlation between TBS categories and final histologic subtypes. Higher Bethesda categories were strongly associated with aggressive histologic variants of papillary thyroid carcinoma (PTC), including tall-cell and other high-risk variants. Notably, over 80% of tall-cell variant PTCs and other aggressive variants were classified as TBS VI on FNA cytology. In contrast, lower Bethesda categories (III and IV) were more frequently associated with follicular variant PTC and minimally invasive follicular thyroid carcinoma, which generally exhibit a more indolent clinical course.

Interestingly, malignancies initially classified as TBS III demonstrated a heterogeneous biological behavior. While many TBS III malignancies were low-risk cancers, a subset exhibited aggressive features, including solid variant PTC and lymph node metastasis. This finding aligns with previous reports suggesting that the heterogeneity of the AUS category contributes to its unpredictable clinical behavior(27, 28). In contrast, TBS IV malignancies were more likely to represent follicular-patterned tumors, including widely invasive follicular thyroid carcinoma and poorly differentiated carcinoma, explaining the modest but meaningful increase in high-risk malignancy observed in this category.

Beyond histologic subtype, we demonstrated that increasing TBS category was significantly associated with established adverse prognostic features, including extrathyroidal extension, multifocality, lymphovascular invasion, and lymph node metastasis. Most of thyroid cancers with central and lateral lymph node metastases were classified as TBS VI, while such features were uncommon in TBS III and IV cytology. Furthermore, the proportion of intermediate- and high-risk cancers, as defined by AJCC and ATA risk stratification, increased progressively from AUS to FN, SM, and malignant categories.

These findings underscore the clinical relevance of cytologic classification in preoperative risk assessment. Incorporating Bethesda category into surgical decision-making may help refine the extent of initial surgery, lymph node evaluation, and postoperative management strategies.

## Declarations

The study was approved by the Internal Review and the Ethics Board of the Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University.

## Contribution statement

Jiixin Xie: Writing – original draft (lead); review and editing (equal). Ying Liu: Conceptualization (lead); Writing – original draft (equal); formal analysis (lead). Wei Ding: Software (lead); writing – review and editing (equal); Dingyuan Luo: Conceptualization (supporting); Writing – original draft (supporting); Writing – review and editing (lead); Methodology (lead)

### Consent for publication

Not Applicable.

### Competing interests

The authors declare no competing interests.

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None.

## Data Availability

All data can be provided as needed. If someone wants to request the data/or have queries from this study, please contact Dingyuan Luo, E-mail: [luody2080@163.com](mailto:luody2080@163.com) , dygluo@ucdavis.edu

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## Tables

TABLE 1. THE SONOGRAPHIC FEATURES AND CYTOLOGY DIAGNOSIS FOR SURGICAL PATIENTS

| Characteristics                   | Total      | Cytology diagnosis |           |           |           |           |            |
|-----------------------------------|------------|--------------------|-----------|-----------|-----------|-----------|------------|
|                                   |            | Nondiagnostic      | Benign    | AUS       | FN        | SM        | Malignant  |
| <b>Number (%)</b>                 | 2960       | 28(0.8)            | 168(5.2)  | 423(13.1) | 292(2.7)  | 141(8.3)  | 1908(69.9) |
| <b>Age(years) *</b>               | 44.5±13.0  | 47.7±12.8          | 46.1±12.9 | 45.4±12.8 | 46.1±14.6 | 41.4±11.8 | 40.8±12.5  |
| <b>Gender (%) *</b>               |            |                    |           |           |           |           |            |
| Male                              | 572(19.3)  | 9(32.1)            | 30(17.9)  | 78(18.4)  | 59(20.2)  | 27(19.1)  | 369(19.3)  |
| Female                            | 2388(80.7) | 19(67.9)           | 138(82.1) | 345(81.6) | 233(79.8) | 114(80.9) | 1539(80.7) |
| <b>Nodule size (mm) *</b>         | 15.2±9.7   | 10.3±3.6           | 18.9±8.8  | 13.8±5.1  | 13.4±7.3  | 15.3±8.7  | 19.6±9.5   |
| <b>Nodule Composition (%) #</b>   |            |                    |           |           |           |           |            |
| Solid                             | 2695(91.1) | 13(46.4)           | 84(50.0)  | 333(78.7) | 268(91.8) | 121(85.8) | 1876(98.3) |
| Mainly solid                      | 229(7.7)   | 5(17.9)            | 68(40.5)  | 87(20.6)  | 22(7.5)   | 18(12.8)  | 29(1.5)    |
| Mainly cystic                     | 36(1.2)    | 10(35.7)           | 16(9.5)   | 3(0.7)    | 2(0.7)    | 2(1.4)    | 3(0.2)     |
| <b>Calcification (%) #</b>        |            |                    |           |           |           |           |            |
| No                                | 385(13.0)  | 13(46.4)           | 90(53.6)  | 119(28.1) | 63(21.6)  | 10(7.1)   | 90(4.7)    |
| Macrocalcification                | 742(25.1)  | 10(35.7)           | 65(38.7)  | 139(32.9) | 93(31.8)  | 21(14.9)  | 414(21.7)  |
| Microcalcification                | 1833(61.9) | 5(17.9)            | 13(7.7)   | 165(39.0) | 136(46.6) | 110(78.0) | 1404(73.6) |
| <b>Sonographic features (%) #</b> |            |                    |           |           |           |           |            |
| Hyperechoic                       | 304(10.3)  | 4(14.3)            | 121(72.1) | 115(27.2) | 50(17.1)  | 9(6.4)    | 5(0.3)     |
| Isoechoic                         | 345(11.6)  | 8(28.5)            | 34(20.2)  | 161(38.1) | 95(32.5)  | 23(16.3)  | 24(1.2)    |
| Hypoechoic                        | 2193(74.1) | 11(39.3)           | 12(7.1)   | 128(30.2) | 133(45.6) | 96(68.1)  | 1813(95.0) |
| Very hypoechoic                   | 118(4.0)   | 5(17.9)            | 1(0.6)    | 19(4.5)   | 14(4.8)   | 13(9.2)   | 66(3.5)    |
| <b>Shape (%) #</b>                |            |                    |           |           |           |           |            |
| Taller-than-wide                  | 2125(71.8) | 12(42.9)           | 98(58.3)  | 162(38.3) | 123(42.1) | 103(73.0) | 1627(85.3) |
| Wider-than-tall                   | 835(28.2)  | 16(57.1)           | 70(41.7)  | 261(61.7) | 169(57.9) | 38(27.0)  | 281(14.7)  |
| <b>ACR TIRADS category (%) #</b>  |            |                    |           |           |           |           |            |
| 3                                 | 137(4.6)   | 16(57.1)           | 76(45.2)  | 6(1.4)    | 21(7.2)   | 7(5.0)    | 11 (0.6)   |
| 4                                 | 1727(58.3) | 11(39.3)           | 85(50.6)  | 389(92.0) | 178(61.0) | 73(51.8)  | 991(51.9)  |
| 5                                 | 1096(37.1) | 1(3.6)             | 7(4.2)    | 28(6.6)   | 93(31.8)  | 61(43.2)  | 906(47.5)  |

AUS, Atypia of undetermined significance; FN, Follicular neoplasm; SM, Suspicious for malignancy

\*Not significant, Calculated using one-way analysis of variance

# $p < 0.05$  for significant differences, Calculated using the chi-square test or the Fisher exact test for categorical variables

TABLE 2. MALIGNANT HISTOLOGIC TYPE FOR SURGICAL PATIENTS BASED ON BETHESDA CATEGORIES AUS, FN, SM AND MALIGNANT

| Malignant<br>Histologic type (%)       | Cytology classification |                 |                 |                   | P value <sup>#</sup> |
|----------------------------------------|-------------------------|-----------------|-----------------|-------------------|----------------------|
|                                        | AUS                     | FN              | SM              | Malignant         |                      |
| <b>Total:2310</b>                      | <b>168(7.3)</b>         | <b>139(6.0)</b> | <b>110(4.8)</b> | <b>1893(81.9)</b> |                      |
| <b>PTC:</b>                            |                         |                 |                 |                   |                      |
| Classic variant                        | 42(25.0)                | 20(14.4)        | 12(10.9)        | 1343(70.9)        | <0.0001              |
| Follicular variant                     | 81(48.2)                | 76(54.7)        | 13(11.8)        | 120(6.3)          | <0.0001              |
| Tall-cell variant                      | 12(7.1)                 | 0(0)            | 18(16.4)        | 136(7.2)          | <0.0001              |
| Other variants                         | 10(6.0)                 | 2(1.4)          | 23(20.9)        | 140(7.4)          | <0.0001              |
| <b>FTC:</b>                            |                         |                 |                 |                   |                      |
| Minimally invasive                     | 21(12.5)                | 33(23.7)        | 17(15.5)        | 21(1.1)           | <0.0001              |
| Encapsulated angioinvasive             | 1(<1)                   | 1(<1)           | 3(2.7)          | 1(<1)             | <0.0001              |
| Widely invasive                        | 1(<1)                   | 7(5.0)          | 3(2.7)          | 17(<1)            | <0.0001              |
| <b>Poorly differentiated carcinoma</b> | 0(0)                    | 0(0)            | 5(4.5)          | 28(1.5)           | 0.0071               |
| <b>Medullary thyroid carcinoma</b>     | 0(0)                    | 0(0)            | 7(6.4)          | 39(2.1)           | 0.0007               |
| <b>Anaplastic thyroid carcinoma</b>    | 0(0)                    | 0(0)            | 5(4.5)          | 14(<1)            | <0.0001              |
| <b>Non-thyroid malignancy</b>          | 0(0)                    | 0(0)            | 4(3.6)          | 34(1.8)           | 0.0440               |

AUS, Atypia of undetermined significance; FN, Follicular neoplasm; SM, Suspicious for malignancy; PTC, Papillary thyroid carcinoma; FTC, Follicular thyroid carcinoma

<sup>#</sup> $p < 0.05$  for significant differences, calculated using the chi-square test or the Fisher exact test for categorical variables

TABLE 3. AGGRESSIVE FEATURES AND RISK STRATIFICATION FOR BETHESDA CATEGORIES AUS, FN, SM AND MALIGNANT

| Prognostic factors and<br>risk stratification (%) | Cytology classification |                 |                 |                   | P value <sup>#</sup> |
|---------------------------------------------------|-------------------------|-----------------|-----------------|-------------------|----------------------|
|                                                   | AUS                     | FN              | SM              | Malignant         |                      |
| <b>Total:2310</b>                                 | <b>168(7.3)</b>         | <b>139(6.0)</b> | <b>110(4.8)</b> | <b>1893(81.9)</b> |                      |
| <b>Aggressive Features:</b>                       |                         |                 |                 |                   |                      |
| Extrathyroidal extension                          | 25(14.9)                | 13(9.4)         | 18(16.4)        | 419(22.1)         | 0.0004               |
| Multifocal disease                                | 27(16.1)                | 8(5.8)          | 12(10.9)        | 329(17.4)         | 0.0016               |
| Lymph vascular invasion                           | 15(8.9)                 | 14(8.3)         | 23(13.7)        | 508(26.8)         | <0.0001              |
| Central lymph node metastasis                     | 46(27.4)                | 33(23.7)        | 24(21.8)        | 675(35.7)         | 0.0002               |
| Lateral lymph node metastasis                     | 11(6.5)                 | 12(8.6)         | 17(15.5)        | 265(14.0)         | 0.0144               |
| <b>Risk stratification:</b>                       |                         |                 |                 |                   |                      |
| Lower-risk cancer                                 | 81(48.2)                | 76(54.7)        | 13(11.8)        | 120(6.3)          | <0.0001              |
| Intermediate-risk cancer                          | 65(38.7)                | 55(39.6)        | 45(40.9)        | 1394(73.6)        | <0.0001              |
| Higher-risk cancer                                | 22(13.1)                | 8(5.7)          | 52(47.3)        | 379(20.1)         | <0.0001              |

AUS, Atypia of undetermined significance; FN, Follicular neoplasm; SM, Suspicious for malignancy

<sup>#</sup> $p < 0.05$  for significant differences, calculated using the chi-square test or the Fisher exact test for categorical variables

Figures

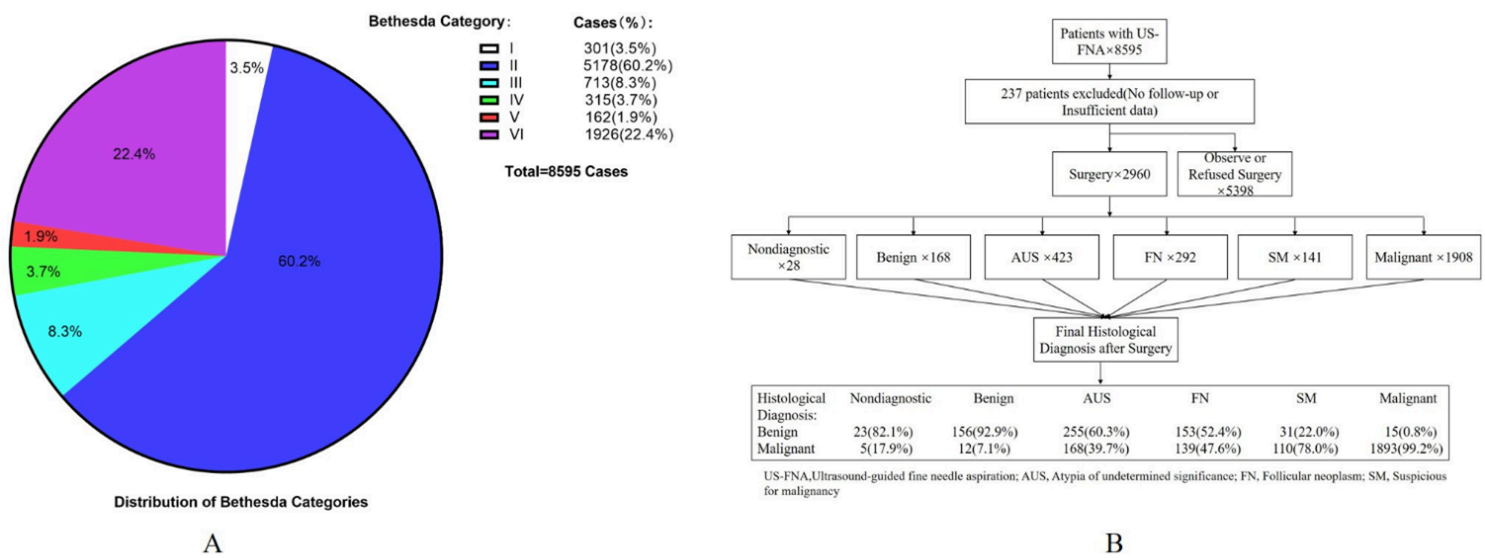


Figure 1

Distribution of Bethesda Categories and Research Flowchart

(A) Bethesda category II (benign) was most common (60.2%), followed by category VI (malignant, 22.4%), category III (AUS, 8.3%), category IV (FN, 3.7%), category I (nondiagnostic, 3.5%), and category V (SM, 1.9%). (B) Flow diagram of patient selection and cytology–histology correlation. A total of 2960 patients underwent surgery and were stratified by Bethesda category, with final histopathological diagnoses classified as benign or malignant.

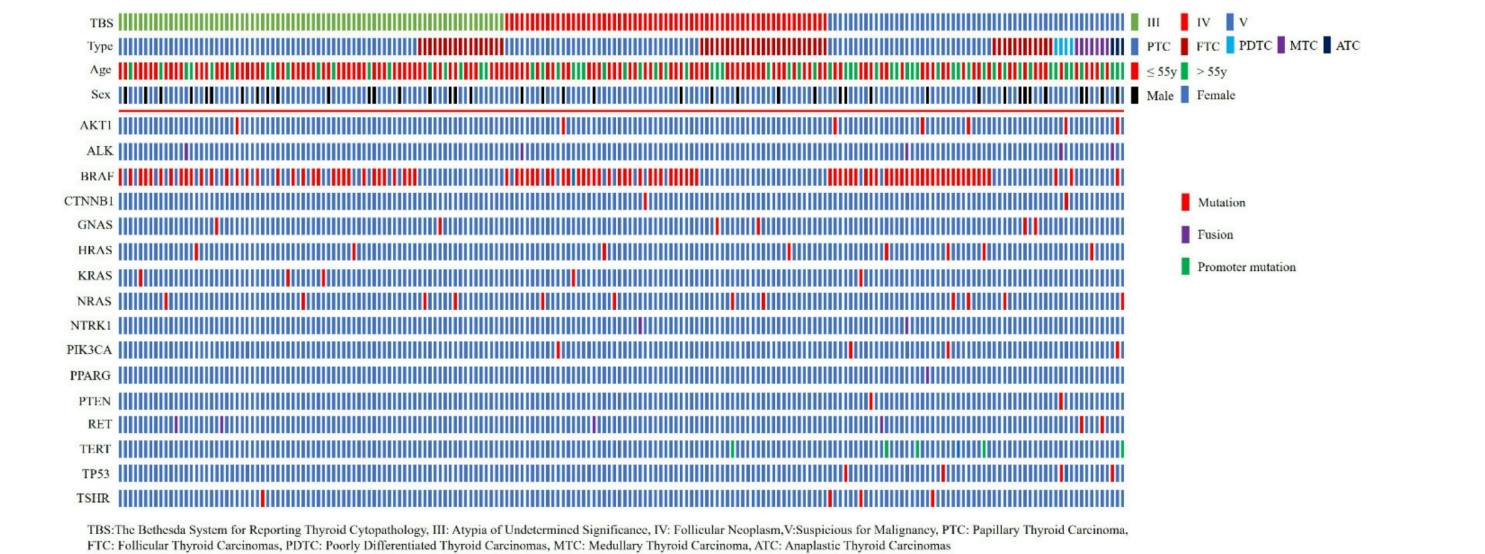


Figure 2

**Genomic Landscape and Clinical Features of Thyroid Tumors**

Genetic alterations and clinicopathological characteristics of thyroid tumors are showed. Each column represents an individual patient. Rows indicate Bethesda System for Reporting Thyroid Cytopathology (TBS) categories, histological subtypes, age, sex, and gene alterations. Distinct molecular patterns are observed across Bethesda categories and tumor subtypes.