

Clinicopathologic and Molecular Characteristics of Pituitary Neuroendocrine Tumors (PitNETs) Treated with Extra-Pseudocapsule Resection and Their Clinical Implications: A Single-Center Experience with 274 Cases

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Abstract

Purpose

To evaluate associations between transcription factor (TF)-defined molecular lineage and the clinical characteristics of pituitary neuroendocrine tumors (PitNETs).

Methods

A retrospective cohort analysis was performed in 274 patients undergoing extra-pseudocapsular transsphenoidal resection, tumors were classified by TF-defined lineage and invasiveness (0–3) and Knosp score. Group differences were tested with appropriate parametric/nonparametric and χ^2 /Fisher's exact methods. Predictors of GTR were examined using prespecified hierarchical multivariable logistic regression, and model discrimination compared using ROC/AUC with LRT and DeLong testing.

Results

TF-defined lineage classification improved diagnostic precision. Among tumors previously classified as nonfunctioning adenomas, 74.7% received a definitive lineage assignment. TF-negative and multilineage tumors showed higher invasiveness rates and higher invasiveness grades than single-lineage tumors. The PIT1–GH/PRL subgroup had the highest prevalence and grade of invasiveness; SOX2 positivity was most frequent in this subgroup (41.7%), which also exhibited the lowest GTR rate (58.3%). SOX2-positive tumors were associated with higher preoperative ACTH and GH levels. SOX2 positivity was more common in multilineage tumors (20.5%) and recurrent cases (20.0%). Compared with Trouillas grading alone, incorporation of invasiveness grade and intraoperative features (capsule status and vascularity) improved discrimination for predicting GTR (AUC, 0.866 vs 0.795; Δ AUC = 0.071; DeLong 95% CI, 0.022–0.120; P = 0.0047).

Conclusions

The 2022 WHO TF-defined lineage system improves diagnostic precision and facilitates interpretation of PitNET differentiation and molecular pathology. Tumors in the PIT1–GH/PRL subgroup demonstrate more aggressive invasive features. SOX2 positivity has the highest proportion in multilineage tumors and in recurrent surgical patients, and close follow-up is needed.

1. Introduction

Since the late 1990s, this center has adopted the diagnostic and therapeutic protocols developed by the Departments of Neurosurgery and Pathology at Erlangen–Nürnberg University (Germany). A pituitary adenoma classification framework based on an immunohistochemical hormone panel (GH, PRL, ACTH, TSH, FSH/LH, and the α -subunit, among others) was implemented to characterize clinical heterogeneity—particularly differences in invasiveness—between functioning and nonfunctioning tumors^{1,2}. In 2022, the World Health Organization (WHO) updated the Classification of Central Nervous System (CNS) Tumors. This revision renamed pituitary adenoma as pituitary neuroendocrine tumors (PitNETs) and incorporated transcription factors (such as PIT1, TPIT, SF1, GATA3, ER α) into the formal classification of PitNET, promoting pituitary neuroendocrine tumor pathological classification toward a more precise, molecular-based framework^{3,4}.

Notably, the updated classification continues to emphasize tumor invasiveness. Accordingly, our center has maintained a focus on invasiveness and differentiation-related features, which represent key phenotypes of aggressive PitNETs. Since 2011, extra-pseudocapsular gross total resection has been adopted⁵, with subsequent improvement in clinical outcomes⁶. In 2019, SOX2 was introduced as a stemness-associated marker in studies of plurihormonal tumors to support assessment of multilineage differentiation⁷. Since 2023, the WHO lineage-based diagnostic criteria have been implemented in parallel with the pre-existing diagnostic system; however, the associations between TF-defined lineage (and related molecular features) and clinical characteristics under the updated framework have not been systematically summarized. Therefore, the present study retrospectively analyzed PitNET cases treated since 2023 to examine the relationship between molecular pathological features in the updated lineage classification and the clinical characteristics and outcomes, with the aim of informing neurosurgical decision-making in routine practice.

2. Materials and Methods

2.1 Study population

Between 2023 and 2025, 305 consecutive transsphenoidal resections for pituitary neuroendocrine tumors (PitNETs) were performed at this center using microscopic or endoscopic extra-pseudocapsular techniques. 294 transsphenoidal resections for PitNETs were performed, including 274 primary surgeries and 20 reoperations for recurrent tumors. The primary-surgery cohort (n = 274) was used for the main analyses. Recurrent cases (n = 20) were analyzed separately as an exploratory cohort. Exclusion criteria for the primary-surgery cohort included incomplete follow-up, postoperative MRI not meeting prespecified assessment standards, and age < 18 years.

2.2 Baseline characteristics

Clinical variables included age, sex, maximum tumor diameter (cm), pituitary-axis hormone levels, pseudocapsule integrity, intraoperative dural invasion findings, radiological invasion, tumor texture, intraoperative vascularity, extent of resection, and endocrine outcome (hormonal remission). Pathological variables included immunohistochemical markers specified by the updated classification (PIT1, TPIT, SF1, GATA3, ER α , Ki-67, and SOX2) and histological evidence of dural invasion.

2.3 PitNET classification criteria

PitNETs were classified according to the WHO *Classification of Tumors of the Central Nervous System* (5th edition) based on lineage-defining transcription factor (TF) immunoreactivity. Tumors were assigned to the PIT1, TPIT, or SF1 lineage according to expression of PIT1, TPIT, and SF1, respectively: the PIT1 lineage included GH-, PRL-, and TSH-secreting tumors and mixed PIT1-lineage subtypes; the TPIT lineage corresponded to ACTH-secreting tumors; and the SF1 lineage corresponded to gonadotroph tumors (FSH and/or LH positive). TF-negative tumors were defined by absence of PIT1, TPIT, and SF1 immunoreactivity. Tumors expressing TFs from more than one lineage were categorized as multilineage.

Given prior work on plurihormonal PitNETs, SOX2 was evaluated as a stemness-associated marker⁷. SOX2 immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections (4 μ m). After deparaffinization and rehydration, heat-induced antigen retrieval was conducted using Tris-EDTA (pH 9.0). Endogenous peroxidase was blocked, and sections were incubated overnight at 4°C with an anti-SOX2 primary antibody (Abcam; rabbit monoclonal; clone EPR3131; ab92494; 1:100), followed by polymer-based HRP detection with DAB chromogen, hematoxylin counterstaining, and mounting. Only nuclear staining in tumor cells was considered positive. The proportion of SOX2-positive tumor nuclei was scored as follows: 0 (0%); 1 (scattered positive cells); 2 (1–5%); 3 (5–10%); 4 (10–30%); 5 (30–50%); 6 (> 50%). A score ≥ 2 ($\geq 1\%$) was defined as SOX2-positive. Scoring was performed independently by two blinded pathologists; discrepancies were resolved by joint review and adjudication by a senior pathologist.

2.4 Invasiveness grading

Tumor invasiveness was evaluated using a three-domain framework (radiological, surgical, and histological evidence) according to Lu et al. Radiological invasiveness was assessed on coronal gadolinium-enhanced T1-weighted MRI and was defined by any imaging evidence of extrasellar invasive growth, including (i) cavernous sinus invasion, graded by the Knosp system (Knosp 3–4); (ii) suprasellar invasion beyond the diaphragma sellae; and/or (iii) invasion/extension into the sphenoid sinus. Surgical invasiveness was defined as macroscopic invasive or destructive tumor behavior observed under direct endoscopic/microscopic visualization, including invasion into the cavernous sinus compartment (beyond the medial wall) and/or destructive extension beyond the sella (e.g., invasion into the sphenoid sinus and/or involvement/disruption of the sellar diaphragm). Histological invasiveness was defined as microscopic infiltration of, and/or destructive invasion into, the basal sellar dura (endosteum) by tumor cells on hematoxylin–eosin–stained sections. Based on these three criteria, tumors were assigned an invasiveness grade as follows: grade 0, none of the three criteria present; grade 1, any one criterion present; grade 2, any two criteria present; and grade 3, all three criteria present⁸.

2.5 Extent of resection and hormonal assessment

Tumor size was measured on coronal gadolinium-enhanced T1-weighted MRI, using the maximal diameter. Postoperative contrast-enhanced MRI was routinely performed at approximately 3 months after surgery (8–12 weeks) to assess residual tumor and extent of resection. Gross total resection (GTR) was defined as absence of residual enhancing tumor on postoperative MRI. Near-total resection (NTR) was defined as suspicious residual enhancement or residual tumor volume < 10% of the initial volume. Subtotal resection (STR) was defined as residual tumor volume > 10% of the initial volume⁹. Pituitary-axis hormone levels were assessed within 7 days preoperatively and on postoperative day 1 to characterize perioperative endocrine status and early biochemical changes.

2.6 Statistical analysis

Analyses were performed using R (version 4.2.0). Continuous variables are presented as mean $\pm$ standard deviation (SD) or median (interquartile range [IQR]), as appropriate. Two-group comparisons used Welch's t-test or the Wilcoxon rank-sum test. Comparisons across three or more groups used one-way analysis of variance (ANOVA) or the Kruskal–Wallis test. Categorical variables are presented as n (%) and compared using Pearson's χ^2 test; Fisher's exact test was applied to 2 $\times$ 2 tables when expected counts were < 5. For multi-category contingency tables with sparse expected counts, P values were estimated using Monte Carlo χ^2 simulations (two-sided; 10,000 permutations); additional Monte Carlo permutation tests were performed for selected overall comparisons, as specified in the table notes. Ordinal outcomes (e.g., invasiveness grade and Knosp grade) were compared using the two-sided Mann–Whitney U test.

To evaluate predictors of GTR (resection grade 2), four prespecified hierarchical multivariable logistic regression models were fitted. Model 1 included age, sex, maximum tumor diameter, proliferative status (Ki-67 $\geq 3\%$ and/or P53 positivity), and imaging invasiveness. Model 2 replaced imaging invasiveness with invasiveness grade ≥ 2 . Model 3 additionally included pseudocapsule integrity and intraoperative vascularity, and Model 4 further included SOX2 status. Results are reported as adjusted odds ratios (aORs) with 95% confidence intervals (CIs). Discrimination was assessed using receiver operating characteristic (ROC) curves and the area under the ROC curve (AUC); AUCs were compared using DeLong's test, and nested models were compared using likelihood ratio tests (LRTs). All tests were two-sided, and P < 0.05 was considered statistically significant.

3. Results

3.1 Clinical characteristics of patients

Among 274 PitNETs, 74.7% of tumors previously classified as hormone-immunonegative under the former diagnostic scheme received a definitive TF-defined lineage assignment (Fig. 1). Lineage distribution was as follows: SF1-only (n = 95), PIT1-only (n = 60), TPIT-only (n = 53), PIT1 + SF1 (n = 32), TF-negative (n = 22), and rare mixed lineages (SF1 + TPIT, n = 7; PIT1 + TPIT, n = 3; triple-lineage, n = 2). Sex distribution was balanced (female, 136/274; male, 138/274). Age and maximum tumor diameter differed across lineages (both $P < 0.001$). Patients with PIT1-only tumors were younger than those with SF1-only tumors ($q < 0.0001$), and PIT1-only tumors were smaller than SF1-only and TF-negative tumors (both $q < 0.0001$). Clinical manifestations linked to hormone axes demonstrated lineage-related enrichment: acral/facial changes were more frequent in PIT1-related lineages, whereas centripetal obesity was more common in the TPIT-only lineage ($P = 0.008$) (Table 1; Supplementary Table 1).

Table 1
Demographic and clinical characteristics stratified by transcription factor lineage.

Variable	SF1-only (n = 95)	PIT1-only (n = 60)	TPIT-only (n = 53)	PIT1 + SF1 (n = 32)	TF- negative (n = 22)	SF1 + TPIT (n = 7)	PIT1 + TPIT (n = 3)	Triple (n = 2)	Overall (N = 274)	P value (Monte Carlo)
Total, n	95	60	53	32	22	7	3	2	274	
Female, n (%)	24 (25.3%)	38 (63.3%)	43 (81.1%)	18 (56.2%)	6 (27.3%)	3 (42.9%)	2 (66.7%)	2 (100.0%)	136 (49.6%)	< 0.001
Male, n (%)	71 (74.7%)	22 (36.7%)	10 (18.9%)	14 (43.8%)	16 (72.7%)	4 (57.1%)	1 (33.3%)	0 (0.0%)	138 (50.4%)	
Age (years), mean $\pm$ SD	52.8 $\pm$ 12.0	40.6 $\pm$ 13.7	50.3 $\pm$ 11.7	49.4 $\pm$ 12.6	49.0 $\pm$ 16.1	49.6 $\pm$ 10.5	44.3 $\pm$ 21.6	30.0 $\pm$ 2.8	48.6 $\pm$ 13.6	< 0.001
Tumor maximum diameter (cm), mean $\pm$ SD	2.6 $\pm$ 0.9	1.8 $\pm$ 0.9	2.4 $\pm$ 1.1	1.9 $\pm$ 0.9	3.0 $\pm$ 1.1	2.1 $\pm$ 1.1	2.2 $\pm$ 0.3	1.5 $\pm$ 0.7	2.3 $\pm$ 1.0	< 0.001
Clinical presentation										
Headache, n (%)	33 (34.7%)	24 (40.0%)	19 (35.8%)	13 (40.6%)	6 (27.3%)	2 (28.6%)	1 (33.3%)	0 (0.0%)	98 (35.8%)	0.923
Dizziness, n (%)	20 (21.1%)	4 (6.7%)	14 (26.4%)	5 (15.6%)	2 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	45 (16.4%)	0.087
Nausea/vomiting, n (%)	7 (7.4%)	0 (0.0%)	0 (0.0%)	1 (3.1%)	2 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	10 (3.6%)	0.205
Decreased visual acuity, n (%)	44 (46.3%)	15 (25.0%)	22 (41.5%)	8 (25.0%)	11 (50.0%)	3 (42.9%)	1 (33.3%)	0 (0.0%)	104 (38.0%)	0.078
Blurred vision, n (%)	1 (1.1%)	2 (3.3%)	4 (7.5%)	1 (3.1%)	3 (13.6%)	2 (28.6%)	0 (0.0%)	1 (50.0%)	14 (5.1%)	0.011
Incidental detection on routine examination, n (%)	17 (17.9%)	4 (6.7%)	1 (1.9%)	3 (9.4%)	1 (4.5%)	2 (28.6%)	0 (0.0%)	0 (0.0%)	28 (10.2%)	0.054
Physical findings										
Visual field defect, n (%)	22 (23.2%)	5 (8.3%)	11 (20.8%)	4 (12.5%)	9 (40.9%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	52 (19.0%)	0.045
Ptoxis, n (%)	2 (2.1%)	0 (0.0%)	0 (0.0%)	1 (3.1%)	2 (9.1%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	6 (2.2%)	0.133
Acral enlargement, n (%)	0 (0.0%)	16 (26.7%)	1 (1.9%)	9 (28.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	26 (9.5%)	< 0.001
Coarsening of facial features, n (%)	0 (0.0%)	18 (30.0%)	1 (1.9%)	10 (31.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	29 (10.6%)	< 0.001
Centripetal obesity, n (%)	0 (0.0%)	1 (1.7%)	8 (15.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	9 (3.3%)	0.008
Menstrual irregularity/amenorrhea, n (%)	5 (5.3%)	19 (31.7%)	6 (11.3%)	6 (18.8%)	2 (9.1%)	0 (0.0%)	2 (66.7%)	2 (100.0%)	42 (15.3%)	< 0.001
Galactorrhea, n (%)	0 (0.0%)	4 (6.7%)	2 (3.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (2.2%)	0.184
Sexual dysfunction, n (%)	0 (0.0%)	3 (5.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (1.1%)	0.169
Note.										
Baseline demographics, tumor maximum diameter (cm), clinical symptoms, and physical examination findings are summarized across transcription factor (TF)-defined lineage categories. Continuous variables are presented as mean $\pm$ SD and categorical variables as n (%). P values were obtained using Monte Carlo permutation tests (two-sided; 10,000 permutations) for overall comparisons across lineage groups.										

3.2. Invasive characteristics of pathological diagnosis in different lineages

3.2.1. PIT1-GH-PRL has stronger invasiveness

Tumor invasiveness was assessed using our center's invasiveness grading system and Knosp grading. The results showed that 95.9% (47/49) of tumors with Knosp ≥ 3 were labeled as invasiveness grade ≥ 2 . Among tumors with invasiveness grade ≥ 2 , 66.2% (92/139) had Knosp < 3 (Supplementary Table 2). Identification of invasiveness in large tumors was consistent with Knosp, and identification of invasiveness in small tumors was more accurate (Supplementary Table 3). When stratified by TF-defined lineage, TF-negative and multilineage tumors showed higher rates of invasiveness (grade ≥ 2 : 54.5% vs 49.5%) and higher invasiveness grades than single-lineage tumors (Table 2; Supplementary Table 4). Among the six major hormone-defined subtypes, the GH-PRL group exhibited the highest proportion of Knosp ≥ 3 (29.4%) (Fig. 2A–B; Supplementary Table 5). Consistently, the PIT1–GH/PRL subgroup showed a higher invasiveness grade compared with other lineages ($P = 0.026$) (Table 3). Within the TPIT + cohort (densely granulated, $n = 54$; sparsely granulated, $n = 7$), sparsely granulated tumors demonstrated a right-shift toward higher invasiveness and Knosp grades (invasiveness grade, $P = 0.039$; Knosp, $P = 0.011$) (Fig. 2C–D), whereas no analogous difference was observed in PIT1-lineage tumors (Supplementary Fig. 1A–B).

Table 2
Distribution of invasiveness grades across lineage types

Lineage type	Grade 0	Grade 1	Grade 2	Grade 3	P value (Monte Carlo)
SF1-only	9 (9.5%)	34 (35.8%)	34 (35.8%)	18 (18.9%)	0.106
PIT1-only	17 (28.3%)	14 (23.3%)	20 (33.3%)	9 (15.0%)	
TPIT-only	12 (22.6%)	19 (35.8%)	15 (28.3%)	7 (13.2%)	
PIT1 + SF1	3 (9.4%)	11 (34.4%)	10 (31.2%)	8 (25.0%)	
TF-negative	4 (18.2%)	6 (27.3%)	6 (27.3%)	6 (27.3%)	
SF1 + TPIT	2 (28.6%)	1 (14.3%)	2 (28.6%)	2 (28.6%)	
PIT1 + TPIT	1 (33.3%)	0 (0.0%)	2 (66.7%)	0 (0.0%)	
Triple	2 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Note.					
The distribution of invasiveness grade (0–3) is shown for each TF lineage category. Values are presented as n (row %). P values were calculated using a Monte Carlo χ^2 test (two-sided; 10,000 permutations).					

Table 3
Invasiveness (grade ≥ 2) and Knosp status (≥ 3) by lineage subgroup

Variable	Level	Overall	SF1- only (n = 95)	TPIT- only (n = 53)	PIT1 + SF1 (n = 32)	TF- negative (n = 22)	SF1 + TPIT (n = 7)	PIT1 + TPIT (n = 3)	Triple (n = 2)	PIT1- GHPRL (n = 12)	PIT1- only (non- GH- PRL) (n = 48)	P value (MC χ ²)
Invasiveness grade	Invasiveness grade < 2	135 (49.3%)	43 (45.3%)	31 (58.5%)	14 (43.8%)	10 (45.5%)	3 (42.9%)	1 (33.3%)	2 (100.0%)	1 (8.3%)	30 (62.5%)	0.026
	Invasiveness grade ≥ 2	139 (50.7%)	52 (54.7%)	22 (41.5%)	18 (56.2%)	12 (54.5%)	4 (57.1%)	2 (66.7%)	0 (0.0%)	11 (91.7%)	18 (37.5%)	
Knosp grade	Knosp < 3	225 (82.1%)	80 (84.2%)	45 (84.9%)	25 (78.1%)	14 (63.6%)	6 (85.7%)	3 (100.0%)	2 (100.0%)	8 (66.7%)	42 (87.5%)	0.254
	Knosp ≥ 3	49 (17.9%)	15 (15.8%)	8 (15.1%)	7 (21.9%)	8 (36.4%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	4 (33.3%)	6 (12.5%)	
Note.												
The proportions of tumors with invasiveness grade ≥ 2 (vs < 2) and with Knosp grade ≥ 3 (vs < 3) are summarized across lineage subgroups. Values are presented as n (% within column). P values were calculated using a Monte Carlo χ ² test (two-sided; 10,000 permutations).												

Figure 1 Sankey diagram linking hormone-defined subtypes with transcription factor (TF)–defined lineages. Nodes on the left represent TF-defined lineages and nodes on the right represent hormone-defined subtypes; numbers adjacent to nodes indicate tumor counts (n). Band width is proportional to the number of tumors within each TF–hormone combination. Percentages shown on bands indicate the proportion of each downstream hormone-defined subtype within a given TF-defined lineage. Bands are color-coded by TF-defined lineage.

3.2.2 Tumor invasiveness is associated with hard texture and rich vascularity

Based on established “invasive/high-risk PitNET” features^{10–12}, variables potentially associated with invasiveness were examined, including markers of proliferative or stemness-related activity (SOX2, Ki-67, P53), integrity of local anatomical barriers (pseudocapsule integrity), and intraoperative characteristics (tumor texture and vascularity) (Supplementary Table 6). In TF-defined lineage groups with $n \geq 10$, TF-negative tumors were more frequently hypervascular ($P = 0.002$) and more often had a firm texture (Table 4). In contrast, stratification by hormone-defined subtype showed only borderline differences in texture and vascularity (texture, $P = 0.051$; vascularity, $P = 0.050$) (Supplementary Table 7).

Table 4
Biomarkers and invasiveness indices across lineage types.

Variable	Level	Overall	SF1- only (n = 95)	PIT1- only (n = 60)	TPIT- only (n = 53)	PIT1 + SF1 (n = 32)	TF- negative (n = 22)	SF1 + TPIT (n = 7)	PIT1 + TPIT (n = 3)	Triple (n = 2)	P value (MC χ²)
SOX2	SOX2 negative	232 (84.7%)	81 (85.3%)	52 (86.7%)	44 (83.0%)	26 (81.2%)	20 (90.9%)	5 (71.4%)	3 (100.0%)	1 (50.0%)	0.705
	SOX2 positive	42 (15.3%)	14 (14.7%)	8 (13.3%)	9 (17.0%)	6 (18.8%)	2 (9.1%)	2 (28.6%)	0 (0.0%)	1 (50.0%)	
Capsule	No capsule	63 (23.0%)	18 (18.9%)	14 (23.3%)	17 (32.1%)	7 (21.9%)	6 (27.3%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	0.369
	Partial/Intact capsule	211 (77.0%)	77 (81.1%)	46 (76.7%)	36 (67.9%)	25 (78.1%)	16 (72.7%)	7 (100.0%)	3 (100.0%)	1 (50.0%)	
Texture	Hard texture	40 (14.6%)	11 (11.6%)	13 (21.7%)	4 (7.5%)	6 (18.8%)	5 (22.7%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	0.153
	Soft texture	234 (85.4%)	84 (88.4%)	47 (78.3%)	49 (92.5%)	26 (81.2%)	17 (77.3%)	7 (100.0%)	3 (100.0%)	1 (50.0%)	
Vascular	Poor vascularity	242 (88.3%)	83 (87.4%)	59 (98.3%)	44 (83.0%)	32 (100.0%)	15 (68.2%)	4 (57.1%)	3 (100.0%)	2 (100.0%)	0.002
	Rich vascularity	32 (11.7%)	12 (12.6%)	1 (1.7%)	9 (17.0%)	0 (0.0%)	7 (31.8%)	3 (42.9%)	0 (0.0%)	0 (0.0%)	
Ki-67	Ki-67 < 3	184 (67.2%)	69 (72.6%)	39 (65.0%)	34 (64.2%)	23 (71.9%)	12 (54.5%)	4 (57.1%)	2 (66.7%)	1 (50.0%)	0.789
	Ki-67 ≥ 3	90 (32.8%)	26 (27.4%)	21 (35.0%)	19 (35.8%)	9 (28.1%)	10 (45.5%)	3 (42.9%)	1 (33.3%)	1 (50.0%)	
P53	Mutant (1)	18 (6.6%)	7 (7.4%)	2 (3.3%)	3 (5.7%)	2 (6.2%)	3 (13.6%)	0 (0.0%)	1 (33.3%)	0 (0.0%)	0.404
	Wild-type (0)	256 (93.4%)	88 (92.6%)	58 (96.7%)	50 (94.3%)	30 (93.8%)	19 (86.4%)	7 (100.0%)	2 (66.7%)	2 (100.0%)	
Invasiveness grade	Invasiveness grade ≥ 2	139 (50.7%)	52 (54.7%)	29 (48.3%)	22 (41.5%)	18 (56.2%)	12 (54.5%)	4 (57.1%)	2 (66.7%)	0 (0.0%)	0.628
	Invasiveness grade < 2	135 (49.3%)	43 (45.3%)	31 (51.7%)	31 (58.5%)	14 (43.8%)	10 (45.5%)	3 (42.9%)	1 (33.3%)	2 (100.0%)	
Knosp	Knosp ≥ 3	49 (17.9%)	15 (15.8%)	10 (16.7%)	8 (15.1%)	7 (21.9%)	8 (36.4%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	0.393
	Knosp < 3	225 (82.1%)	80 (84.2%)	50 (83.3%)	45 (84.9%)	25 (78.1%)	14 (63.6%)	6 (85.7%)	3 (100.0%)	2 (100.0%)	
Note.											
SOX2 status, pseudocapsule integrity, tumor texture, intraoperative vascularity, Ki-67 category, P53 status, and invasiveness indices (invasiveness grade ≥ 2; Knosp grade ≥ 3) are summarized across TF-defined lineage categories. Values are presented as n (% within column). P values were calculated using a Monte Carlo χ² test (two-sided; 10,000 permutations).											

Figure 2 Invasiveness across tumor subtypes and lineages. (A–B) Distribution of invasiveness grade (0–3) shown by median (dot) and interquartile range (IQR; horizontal line, 25th–75th percentiles); sample size is shown in parentheses. (A) Stratified by hormone-defined subtype. (B) Stratified by TF-defined lineage. Overall distributions were compared using the Kruskal–Wallis test; P values are shown in panel titles. (C–D) Cumulative distribution curves stratified by granulation pattern within the TPIT-related cohort (TPIT-only, SF1 + TPIT, PIT1 + TPIT, and triple-lineage). (C) Invasiveness grade (0–3). The sparsely granulated group shows higher cumulative proportions at severe-grade thresholds; distributions were compared using a two-sided Mann–Whitney U test ($P = 0.039$). (D) Knosp grade (0–4); distributions were compared using a two-sided Mann–Whitney U test ($P = 0.011$). Densely and sparsely granulated tumors are indicated in the legend.

3.3. SOX2-positive expression is higher in multilineage and recurrent patients

Overall, SOX2 positivity was observed in 15.3% of tumors. SOX2 positivity was higher in multilineage tumors than in single-lineage tumors (20.5% vs 14.9%) (Supplementary Table 8). Within single-lineage tumors, the PIT1–GH/PRL subgroup showed the highest SOX2 positivity rate, although the between-lineage difference did not reach statistical significance ($P = 0.089$) (Supplementary Table 8). SOX2-positive tumors were associated with higher preoperative ACTH and GH levels (Supplementary Table 9). Among recurrent cases ($n = 20$), SOX2 positivity was 20.0% (Supplementary Table 10).

3.4. Risk factors affecting gross total resection

In univariable analyses, non-GTR was associated with larger maximum tumor diameter, higher proliferative activity (greater proportion with Ki-67 ≥ 3%), absence of a pseudocapsule, more advanced invasiveness (higher proportions of invasiveness grade ≥ 2 and Knosp ≥ 3), and hypervascularity (all $P <$

0.001). GTR rates differed across lineages, with the lowest rates observed in the PIT1–GH/PRL subgroup and TF-negative tumors (58.3% and 59.1%, respectively) (Table 5).

Table 5
Univariate analysis of factors associated with gross total resection (GTR).

Variable	Level	Non-GTR (n = 75)	GTR (n = 199)	Total (N = 274)	P value	Test
lineage	PIT1-GH-PRL	5 (6.7%)	7 (3.5%)	12 (4.4%)	0.442	Monte Carlo χ^2
	PIT1-only	10 (13.3%)	38 (19.1%)	48 (17.5%)		
	PIT1 + SF1	8 (10.7%)	24 (12.1%)	32 (11.7%)		
	PIT1 + TPIT	0 (0.0%)	3 (1.5%)	3 (1.1%)		
	SF1 + TPIT	2 (2.7%)	5 (2.5%)	7 (2.6%)		
	SF1-only	23 (30.7%)	72 (36.2%)	95 (34.7%)		
	TF-negative	9 (12.0%)	13 (6.5%)	22 (8.0%)		
	TPIT-only	18 (24.0%)	35 (17.6%)	53 (19.3%)		
	Triple	0 (0.0%)	2 (1.0%)	2 (0.7%)		
Hormone type	ACTH	5 (6.7%)	22 (11.1%)	27 (9.9%)	0.634	Monte Carlo χ^2
	GH	2 (2.7%)	5 (2.5%)	7 (2.6%)		
	GH-PRL	6 (8.0%)	11 (5.5%)	17 (6.2%)		
	Gonadotroph	13 (17.3%)	51 (25.6%)	64 (23.4%)		
	Non-functioning	28 (37.3%)	59 (29.6%)	87 (31.8%)		
	PRL	8 (10.7%)	22 (11.1%)	30 (10.9%)		
	Plurihormonal-other	11 (14.7%)	27 (13.6%)	38 (13.9%)		
	TSH	2 (2.7%)	2 (1.0%)	4 (1.5%)		
SOX2_group	SOX2 negative	66 (88.0%)	166 (83.4%)	232 (84.7%)	0.348	Pearson χ^2
	SOX2 positive	9 (12.0%)	33 (16.6%)	42 (15.3%)		
Ki67_group	Ki67 < 3	36 (48.0%)	148 (74.4%)	184 (67.2%)	< 0.001	Pearson χ^2
	Ki67 $\geq$ 3	39 (52.0%)	51 (25.6%)	90 (32.8%)		
Capsule_group	No capsule	34 (45.3%)	29 (14.6%)	63 (23.0%)	< 0.001	Pearson χ^2
	Partial/Intact capsule	41 (54.7%)	170 (85.4%)	211 (77.0%)		
Texture_group	Soft texture	61 (81.3%)	173 (86.9%)	234 (85.4%)	0.242	Pearson χ^2
	Hard texture	14 (18.7%)	26 (13.1%)	40 (14.6%)		
Vascular_group	Rich vascularity	19 (25.3%)	13 (6.5%)	32 (11.7%)	< 0.001	Pearson χ^2
	Poor vascularity	56 (74.7%)	186 (93.5%)	242 (88.3%)		
P53	Wild-type (0)	70 (93.3%)	186 (93.5%)	256 (93.4%)	1.000	Fisher's exact
	Mutant (1)	5 (6.7%)	13 (6.5%)	18 (6.6%)		
Invasiveness grade	< 2	11 (14.7%)	124 (62.3%)	135 (49.3%)	< 0.001	Pearson χ^2
	$\geq$ 2	64 (85.3%)	75 (37.7%)	139 (50.7%)		
Knosp	< 3	43 (57.3%)	182 (91.5%)	225 (82.1%)	< 0.001	Pearson χ^2
	$\geq$ 3	32 (42.7%)	17 (8.5%)	49 (17.9%)		
Granulation	Densely granulated	55 (73.3%)	151 (75.9%)	206 (75.2%)	0.664	Pearson χ^2
	Non-densely granulated (sparsely granulated/negative)	20 (26.7%)	48 (24.1%)	68 (24.8%)		

Note.

Univariable associations between candidate clinical/pathological factors and gross total resection (GTR). Values are shown as n (% within column) for categorical variables and as mean $\pm$ SD for continuous variables. P values are two-sided, and the statistical test used for each variable is indicated in the "Test" column (Monte Carlo χ^2 with 10,000 permutations for multi-category tables; Pearson's χ^2 or Fisher's exact test for 2x2 tables, as appropriate; Welch's t-test for continuous variables).

Variable	Level	Non-GTR (n = 75)	GTR (n = 199)	Total (N = 274)	P value	Test
Sex	Female	34 (45.3%)	102 (51.3%)	136 (49.6%)	0.382	Pearson χ^2
	Male	41 (54.7%)	97 (48.7%)	138 (50.4%)		
Age	mean $\pm$ SD	49.6 $\pm$ 13.7	48.2 $\pm$ 13.5	48.6 $\pm$ 13.6	0.445	Welch's t-test
Tumor maximum diameter (cm)	mean $\pm$ SD	3.0 $\pm$ 1.1	2.1 $\pm$ 0.9	2.3 $\pm$ 1.0	< 0.001	Welch's t-test
Note.						
Univariable associations between candidate clinical/pathological factors and gross total resection (GTR). Values are shown as n (% within column) for categorical variables and as mean $\pm$ SD for continuous variables. P values are two-sided, and the statistical test used for each variable is indicated in the "Test" column (Monte Carlo χ^2 with 10,000 permutations for multi-category tables; Pearson's χ^2 or Fisher's exact test for 2x2 tables, as appropriate; Welch's t-test for continuous variables).						

In multivariable analyses, four prespecified hierarchical logistic regression models were evaluated for prediction of GTR. Referring to the Trouillas stratification concept of "invasiveness + proliferative activity", the AUC of Model 1 (age, sex, tumor size, proliferation [Ki-67 $\geq$ 3% or P53 positive] and imaging invasion) was 0.795. After Model 2 replaced the imaging invasion variable with invasiveness grade ($\geq$ 2), discrimination improved to AUC = 0.828. Adding pseudocapsule integrity and vascularity (Model 3) further improved discrimination (AUC = 0.866) and model fit (LRT: LR χ^2 [df = 2] = 27.773, P < 0.001) (Fig. 3A). Addition of SOX2 status (Model 4) did not improve discrimination (AUC = 0.866) and did not significantly improve model fit (LRT: LR χ^2 [df = 1] = 0.031, P = 0.860) (Supplementary Fig. 3A).

Figure 3 Receiver operating characteristic (ROC) curves for prespecified multivariable logistic regression models predicting gross total resection (GTR; yes/no). Model 1 included age, sex, maximum tumor diameter, proliferative status (Ki-67 $\geq$ 3% and/or P53 positivity), and imaging invasiveness. Model 2 replaced imaging invasiveness with invasiveness grade $\geq$ 2. Model 3 additionally included pseudocapsule integrity (partial/intact vs absent) and intraoperative vascularity (rich vs poor). The diagonal dashed line indicates chance-level discrimination.

4. Discussion

With advances in molecular pathology, cell biology, and epigenetics, the World Health Organization (WHO) updated its Classification of Central Nervous System (CNS) Tumors in 2022. This revision not only recommended renaming pituitary adenoma as pituitary neuroendocrine tumors (pituitary neuroendocrine tumors, PitNETs), but also incorporated transcription factors defining pituitary cell lineages (such as PIT1, TPIT, SF1, GATA3, ER α) into the formal classification of PitNET^{3,4}. Consistent with prior reports, TF-defined lineage assignment improves diagnostic attribution, particularly for tumors previously labeled as nonfunctioning pituitary adenomas. In the present cohort, 74.7% of tumors that were hormone-immunonegative under the former diagnostic scheme received a definitive lineage assignment, aligning with published trends^{4,13}.

Using the invasiveness grading system applied at this center, invasiveness classification showed high concordance with Knosp grading for larger tumors, while identifying additional invasive tumors among smaller lesions. When stratified by hormone-defined subtype, GH–PRL tumors most frequently exhibited high invasiveness grades. Integrating TF-defined lineage further indicated that the PIT1–GH/PRL subgroup tended to show greater invasiveness than other lineages. Moreover, within the TPIT + lineage, the sparsely granulated subtype was associated with higher invasiveness grades and higher Knosp grades. Recent evidence indicates that silent corticotroph adenomas (SCAs) have higher rates of cavernous sinus invasion, lower gross total resection (GTR) rates, and increased recurrence risk¹⁴. Together, these observations underscore substantial heterogeneity even within the same lineage and support refined risk stratification within TF-defined categories¹⁵.

Multilineage tumors demonstrated higher invasiveness rates than single-lineage tumors, consistent with previous studies^{13,163,16}. Importantly, "plurihormonal" and "multilineage" are not interchangeable: under the updated classification, plurihormonal tumors include both multilineage plurihormonal PitNETs and single-lineage plurihormonal PitNETs (e.g., GH/PRL)^{3,4}. Previously, our center found that 33.3% of plurihormonal adenoma patients were SOX2-positive⁷; in this study, overall 15.8% of tumors were SOX2-positive, with higher positivity in multilineage (n > 5). Notably, SOX2 positivity was highest in PIT1–GH/PRL tumors (41.7%), which also showed a relatively low GTR rate (58.3%); these tumors were commonly classified as plurihormonal secretory tumors under the former framework. SOX2 positivity was associated with higher preoperative hormone levels, while no independent association between SOX2 status and GTR was identified in multivariable modeling; however, SOX2 positivity appeared enriched among recurrent cases. Given ongoing inconsistency in the literature regarding associations between SOX2, invasiveness, and prognosis, further studies are warranted to clarify the prognostic significance of SOX2—particularly in plurihormonal PitNETs^{17 18}.

5. Conclusions

The 2022 WHO TF-defined lineage classification improves lineage attribution and facilitates interpretation of PitNET differentiation and molecular pathology. Multilineage PitNETs show a tendency toward greater invasiveness, and the PIT1–GH/PRL subgroup demonstrates more aggressive invasive features together with the highest SOX2 positivity. Although SOX2 was not independently associated with GTR in the current models, its enrichment in plurihormonal and recurrent tumors suggests potential prognostic relevance that warrants validation in larger cohorts with longer follow-up.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

This study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board (Ethics Committee) of Tongji Hospital, Tongji Medical College (No. TJ-IRB20220325). Data were anonymized prior to analysis to protect patient privacy.

Consent to participate

The requirement for informed consent to participate was waived by the Institutional Review Board due to the retrospective nature of the cohort study.

Consent to publish

Not applicable.

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Author Contribution

Conceptualization: X.L., K.Z., and T.L.; Methodology: K.Z.; Software: H.L., L.L.; Validation: H.L., L.L.; Formal analysis: X.L., K.Z.; Investigation: X.L., K.Z.; Resources: T.L., C.K.; Data curation: X.L., K.Z.; Writing—original draft: X.W., J.C., J.W.; Writing—review & editing: X.W., J.W., T.L.; Visualization: X.L.; Supervision: J.W.; Project administration: T.L.; Funding acquisition: T.L. All authors read and approved the final manuscript.

Data Availability

The data that support the findings of this study are included in the article. Further inquiries are available from the corresponding author upon reasonable request.

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Figures

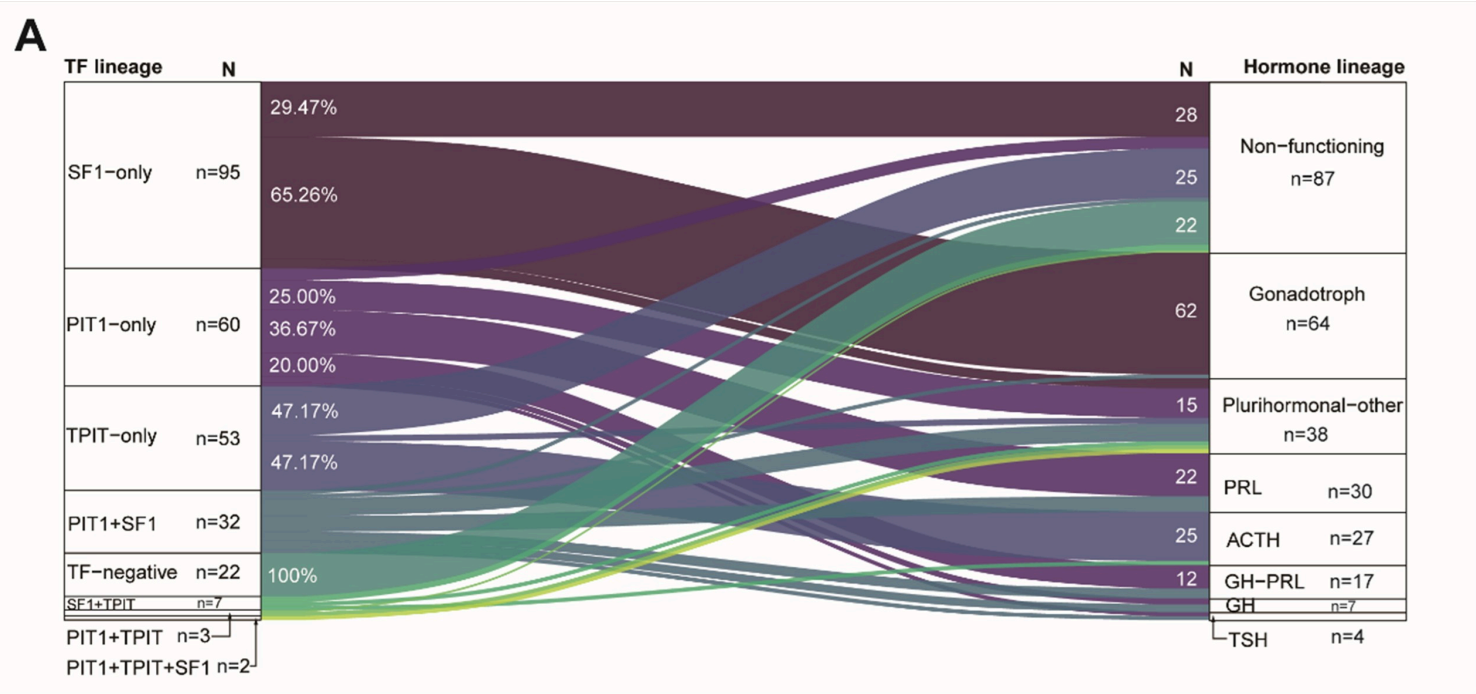


Figure 1

Sankey diagram linking hormone-defined subtypes with transcription factor (TF)-defined lineages. Nodes on the left represent TF-defined lineages and nodes on the right represent hormone-defined subtypes; numbers adjacent to nodes indicate tumor counts (n). Band width is proportional to the number of tumors within each TF-hormone combination. Percentages shown on bands indicate the proportion of each downstream hormone-defined subtype within a given TF-defined lineage. Bands are color-coded by TF-defined lineage.

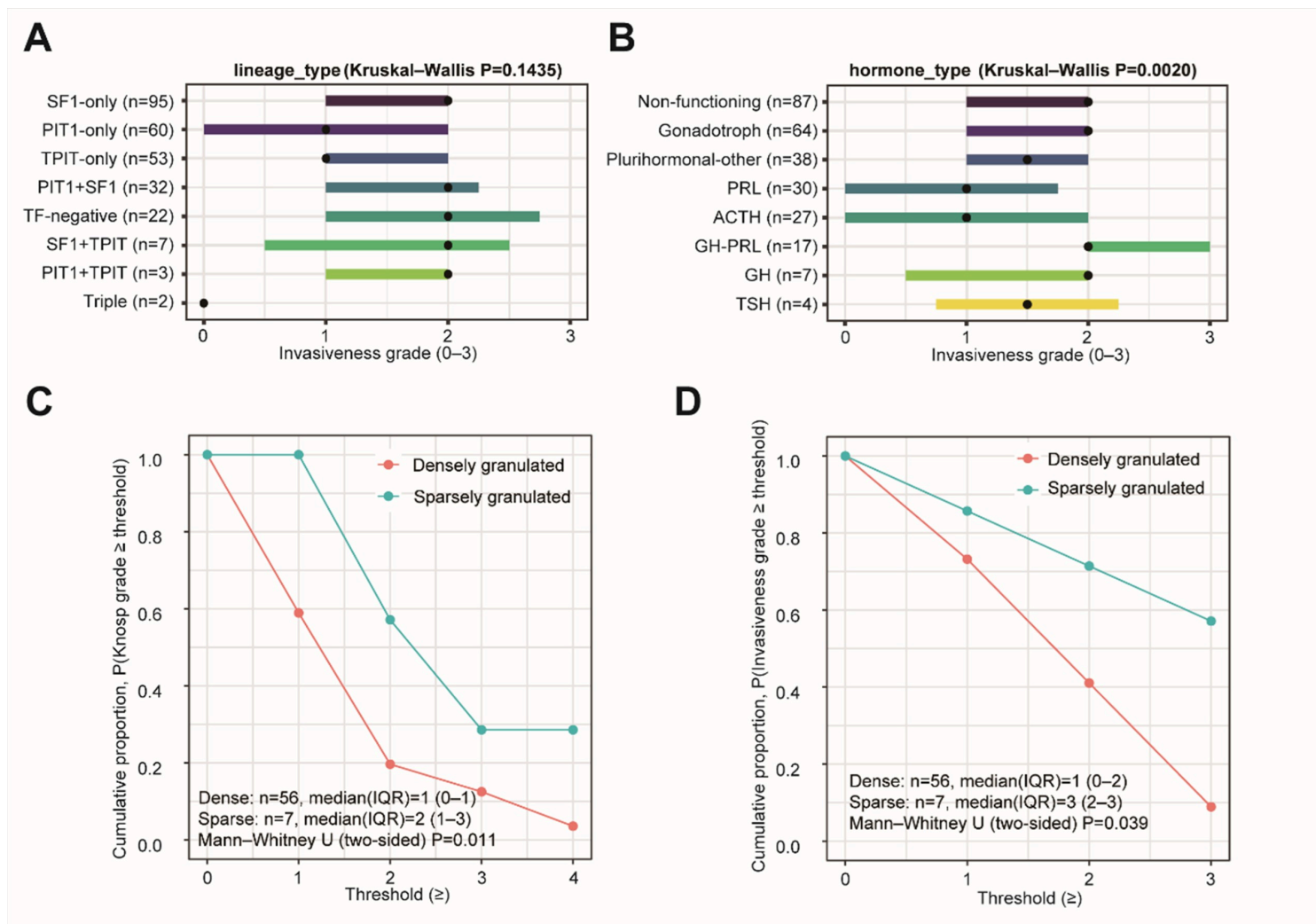


Figure 2

Invasiveness across tumor subtypes and lineages. (A–B) Distribution of invasiveness grade (0–3) shown by median (dot) and interquartile range (IQR; horizontal line, 25th–75th percentiles); sample size is shown in parentheses. (A) Stratified by hormone-defined subtype. (B) Stratified by TF-defined lineage. Overall distributions were compared using the Kruskal–Wallis test; P values are shown in panel titles. (C–D) Cumulative distribution curves stratified by granulation pattern within the TPIT-related cohort (TPIT-only, SF1+TPIT, PIT1+TPIT, and triple-lineage). (C) Invasiveness grade (0–3). The sparsely granulated group shows higher cumulative proportions at severe-grade thresholds; distributions were compared using a two-sided Mann–Whitney U test ($P=0.039$). (D) Knosp grade (0–4); distributions were compared using a two-sided Mann–Whitney U test ($P=0.011$). Densely and sparsely granulated tumors are indicated in the legend.

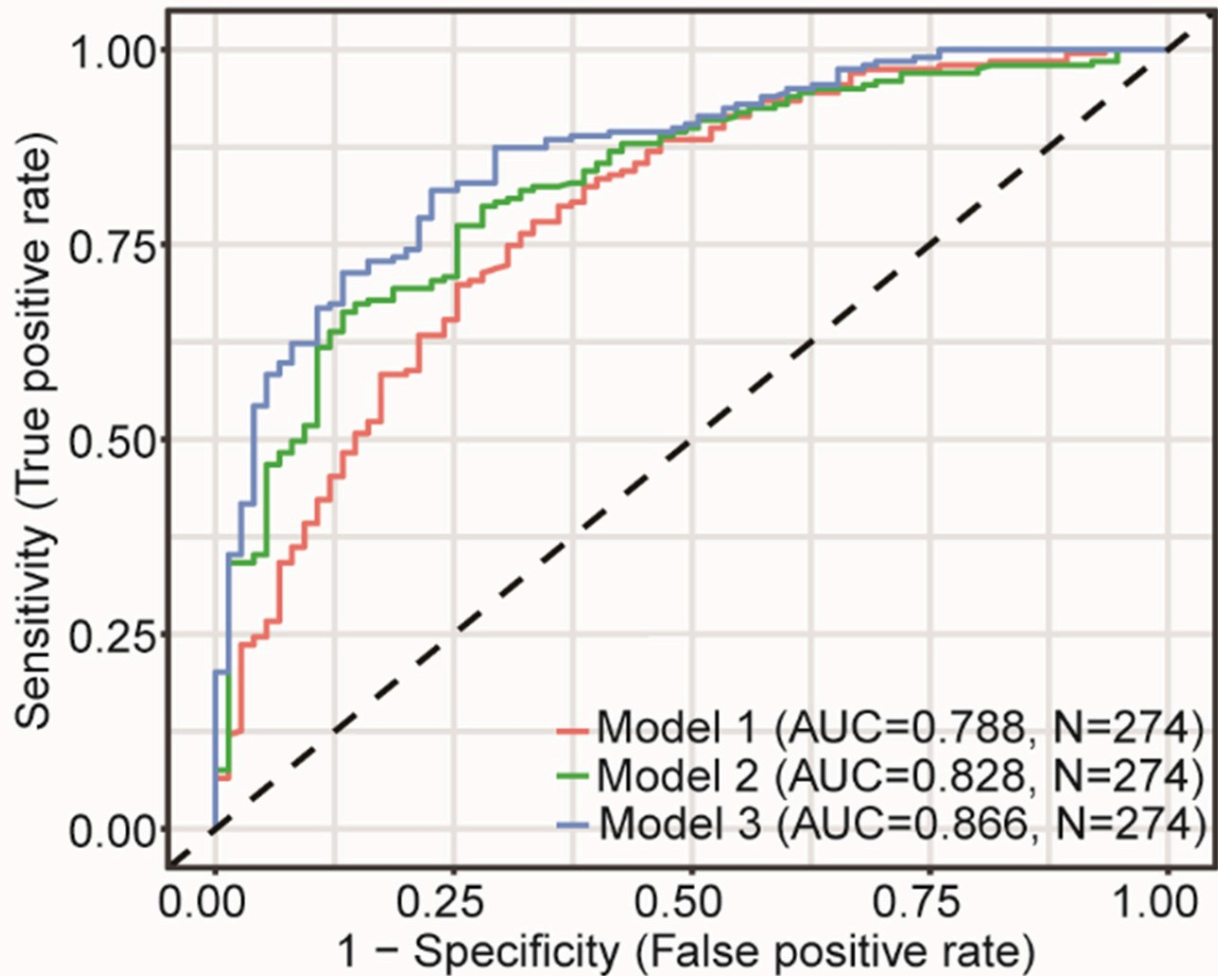
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Figure 3

Receiver operating characteristic (ROC) curves for prespecified multivariable logistic regression models predicting gross total resection (GTR; yes/no). Model 1 included age, sex, maximum tumor diameter, proliferative status (Ki-67 $\geq 3\%$ and/or P53 positivity), and imaging invasiveness. Model 2 replaced imaging invasiveness with invasiveness grade ≥ 2 . Model 3 additionally included pseudocapsule integrity (partial/intact vs absent) and intraoperative vascularity (rich vs poor). The diagonal dashed line indicates chance-level discrimination.

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