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Predictive modeling for pituitary adenomas: single center experience in 501 consecutive patients

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Abstract

Background Personalized postoperative management of patients with pituitary adenomas requires an early risk stratification system.

Methods We reviewed 501 cases operated between 10/27/2011 and 5/5/2016 by a single neurosurgeon. We determined biochemical remission and tumor resection at 3 months, and biochemical recurrence, tumor recurrence, radiation and reoperation during follow-up. We considered age, gender, tumor diameter, cavernous sinus invasion (CSI) by MRI, diagnostic category (clinical, biochemical and immunohistochemical), and proliferation markers in a Cox proportional hazards model. We built predictive models with the significant parameters and used Kaplan–Meier survival curves for time-dependent analyses.

Results The 501 cases comprised 141 functional and 360 nonfunctional adenomas. Tumor diameter, CSI, and ki-67 index predicted long-term events. Model 1 (CSI, diameter ≥ 2.9 cm and ki-67 $> 3\%$) identified 18 (3.6%) adenomas and predicted persistent hypersecretory syndrome and residual tumor with 98.7% specificity (OR 8.6; CI 3.0–24.7). Model 2 (ki-67 $> 3\%$ and CSI) identified 48 (9.6%) adenomas and had 93.1% specificity (OR 3.3; CI 1.8–6.0). Model 3 (ki-67 $> 3\%$, mitoses and p53, former "atypical" adenoma) identified 26 (5.2%) adenomas and had 96.0% specificity (OR 2.3; CI 1.0–5.0). Model 1 best predicted the long-term event-free survival and was strengthened when Knosp 3–4 CSI grades were used. Model 2 better identified the smaller adenomas at risk. Among the WHO 2017 special PA subtypes, patients with silent corticotroph adenoma had a lower event-free survival than ACTH-negative nonfunctional adenomas.

Conclusion Use of CSI, ki-67 and tumor diameter in prediction models facilitates tailored surveillance and management of patients with pituitary adenomas.

Keywords Pituitary adenoma · Cavernous sinus invasion · Ki-67 · Diameter · Atypical adenoma · Aggressive adenoma

Abbreviations

CSI	Cavernous sinus invasion
PPV	Positive predictive value
NPV	Negative predictive value
SD	Standard deviation
CD	Cushing's disease
ACM	Acromegaly
NFA	Non-functional adenoma
SCA	Silent ACTH-positive adenoma
ACTH	Adrenocorticotropic hormone
PA	Pituitary adenomas

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Introduction

Pituitary adenomas (PAs) account for approximately 15% of central nervous system tumors [1]. Transphenoidal adenomectomy (TSA) is the first line treatment for nonfunctional and functional PAs secreting growth hormone (GH) and ACTH [2, 3]. Prolactin-secreting adenomas are generally treated surgically in cases of resistance or intolerance to dopamine agonists or vision loss [4]. Reoperation, radiation and medications are used in patients with

PAs with residual tumor, progression or recurrence, and for persistent or recurrent hormone secretory syndromes. Some PAs are resistant despite multimodal therapy [5–7]. Predicting PA behavior is challenging but worthwhile, as early identification of potentially aggressive tumors may improve outcomes.

In 2004, the World Health Organization (WHO) introduced the category of "atypical" PA defined by three proliferative markers: ki-67 labelling index > 3%, p53 immunopositivity, and increased mitoses [8]. However, several studies did not demonstrate that "atypical" PAs had worse clinical postoperative outcomes than "non-atypical" [9, 10]. On the other hand, preoperative evidence of cavernous sinus invasion (CSI) is a major determinant of incomplete surgical resection which leads to postoperative tumor residual or persistent biochemical disease [11–14]. The updated 2017 WHO classification of PAs discouraged the use of "atypical adenoma" and routine p53 evaluation, and endorsed immunostaining for cell-specific pituitary transcription factors and anterior pituitary hormones [15]. To predict the potential for tumor recurrence, the WHO retained the recommendation to assess ki-67 labelling index and mitotic activity and emphasized the use of clinical parameters such as CSI. The WHO also underlined some special PA subtypes with potentially more aggressive clinical behavior: sparsely granulated (SG) somatotroph adenoma, lactotroph adenoma in men, silent corticotroph adenoma, Crooke cell adenoma, and plurihormonal PIT-1-positive adenoma [15].

The primary aims of our study were to: (1a) evaluate the impact of demographic, radiological and histologic characteristics on postoperative outcomes and events, and (1b) to build predictive models with the significant parameters. Our secondary aim was (2) to evaluate postoperative course in patients with special PA subtypes.

Methods

Patients

We included all patients with PAs operated between October 27, 2011 and May 5, 2016 at Emory University Hospital by a single expert neurosurgeon (N.M.O.) with over 25 years of experience. During this time, we routinely recorded information on demographic, clinical, biochemical, radiological and histological characteristics in the Emory University's Pituitary Tumor Database (REDcap 7.6.9). We ascertained postoperative outcome course and events by retrospective chart review through December 31st, 2017. The study was approved by the Emory University Hospital Institutional Review Board (IRB00019648).

Imaging

We obtained maximum tumor diameter and CSI from radiology reports which we corroborated with the neurosurgeon's preoperative notes. Imaging studies were not all done at our institution because many patients were referred to our tertiary care center after having MRIs elsewhere. All MRIs were directly reviewed by our pituitary neurosurgeon (NMO). CSI was defined by tumor extension beyond the line corresponding to the medial tangents of the two components of the intracavernous internal carotid artery. When available, we included the Knosp grades 1–4 which were based on the direct MRI review by the neurosurgeon (NMO) [16]. Determination of CSI was based on preoperative imaging not intraoperative findings.

Histology

During the study period, neuropathologists at our institution followed the WHO 2004 recommendations for PAs. Immunohistochemistry for pituitary hormones was performed on formalin-fixed, paraffin-embedded tissue to define somatotroph, thyrotroph, lactotroph, corticotroph, gonadotroph, plurihormonal and null cell adenomas. Immunohistochemistry with mouse monoclonal antibodies was used to determine ki-67 labelling index (MIB-1) and p53 positivity (Dako North America, Via Real Carpinteria, CA, USA). The ki-67 labelling index was reported based on a 3% threshold. The p53 immunohistochemistry was described as positive or negative. Increased mitotic activity was determined by surveying multiple high power fields with a qualitative assessment of degree of mitotic activity performed according to WHO 2004 recommendations. Pituitary transcription factors were not routinely performed, as this WHO recommendation emerged in 2017. The immunohistochemical assays were optimized and validated as laboratory-developed tests for routine clinical use in our hospital immunohistochemistry laboratory, using commercially available and widely-implemented antibodies and techniques. The assays were incorporated into the histopathological and immunohistochemical workup for pituitary adenomas at our institution, interpreted by board-certified neuropathologists as a part of clinical practice.

For somatotroph adenomas and other adenomas expressing GH, immunohistochemistry with an anti-CAM 5.2 mouse monoclonal antibody (Beckton Dickinson, Franklin Lakes, NJ, USA) was employed to distinguish densely granulated (DG) and sparsely granulated (SG) adenomas. DG adenomas were defined based on a perinuclear pattern of CAM5.2 immunoreactivity, with few or no fibrous bodies. In contrast, SG adenomas displayed

numerous fibrous bodies in the cytoplasm. Crooke cell adenoma was defined as a corticotroph adenoma with Crooke hyaline changes in numerous neoplastic cells.

Clinical outcomes

Short-term outcomes were determined approximately 3 months postoperatively as follows: biochemical remission in the absence of adjuvant medical treatment for functional PAs and residual tumor evaluated by MRI for NFA. For acromegaly, remission was defined as age- and gender-appropriate insulin like growth factor-1 (IGF-1) levels and GH suppression to oral glucose challenge below 0.4 ng/mL; in patients who did not have GH suppression test, fasting GH < 1 ng/mL was used in conjunction with IGF-1. For Cushing's disease, remission was defined as biochemically-confirmed corticoadrenal insufficiency or normocortisolemia, in addition to normalization of Cushing's screening tests. For prolactinomas, remission was defined as normalized gender-appropriate prolactin levels. For TSH-secreting adenomas, remission was defined as normalized thyroid hormones.

Long-term outcomes and events after surgery included biochemical recurrence (for functional PAs), radiological tumor recurrence, radiation therapy and reoperation. Tumor recurrence during follow-up was defined as the emergence of tumor in the face of a prior negative 3-month MRI (without residual tumor). For patients with NFA, reoperation was considered for surgically-accessible residual tumors on the 3 months postoperative MRI scan, or tumor progression or recurrence during follow-up. Radiation was considered for tumor residual in the cavernous sinuses or its progression during follow-up. Biochemical recurrence was defined as return of hypersecretory syndrome in patients who achieved remission at 3 months postoperatively. For patients with acromegaly who did not achieve biochemical remission, medical treatment with somatostatin receptor ligands was used as first line therapy, with reoperation and radiation recommended in individual cases depending on tumor accessibility, as well as response and tolerance to medical therapy. For patients with Cushing's disease, reoperation was indicated for those who remained hypercortisolemic after surgery. For recurrent hypercortisolemia, reoperation or radiation was recommended depending on the location of the tumor, with medical treatment used as a bridge pending response to radiation, and in those patients who declined radiation or reoperation. Patients with prolactinomas with persistent or recurrent hyperprolactinemia postoperatively were treated with dopamine agonists, while reoperation or radiation were recommended on an individual basis in patients resistant or intolerant to medical therapy.

The vital status of patients as of December 31st, 2017 was determined through search engine PeopleSmart (https://www.peoplesmart.com/) and confirmed through finding the online obituary of the patient. The cause of death could not be ascertained for all patients.

Statistical analysis and modeling

Normality was assessed for all continuous variables and normally-distributed variables were reported as mean and standard deviation (SD). Fischer exact test was used for comparison of categorical variables and t-tests for normally distributed continuous variables. The Kruskal–Wallis test was used for non-normally distributed variables which are reported as median and interquartile range.

Model selection for long-term survival was done using Cox proportional hazards model with forward selection considering age, gender, CSI, Knosp grades, tumor diameter (both as a continuous and a categorical variable), diagnostic category (determined by corroboration of clinical and biochemical presentation with immunohistochemistry results), and proliferation markers as binary covariates (ki-67 index > 3%, p53 immunopositivity, and increased mitoses). The Youden Index calculation was used to determine the predictive cut-off for tumor diameter. Akaike information criterion was used as an estimator of the relative quality of the statistical models to determine the best fitting model [17]. Positive and negative predictive values for short-term outcomes were calculated for each model. Kaplan–Meier curves and the lifetest procedure were used to assess long-term survival for all models.

Statistical significance was defined as $p < 0.05$. Analyses were done using SAS 9.4 statistical software.

Results

Baseline characteristics, histology and postoperative outcomes

Preoperative and histological characteristics of the 141 functional adenomas and 360 NFA's are shown in Table 1. Surgical approach was transsphenoidal in 499 patients and craniotomy in 2 patients with large multi-compartmental tumors. Among the NFA, there were 231 positive by immunohistochemistry for FSH, LH or both, 41 silent ACTH adenomas (SCA), 30 null cell adenomas, 16 silent lactotroph adenomas, 1 silent GH adenoma, 28 with staining for 2 or more anterior pituitary hormones (other than FSH and LH combination) and 13 with rare, weak or equivocal hormone staining. Functional lactotroph type had the largest proportion of tumors with high ki-67 (43.4%).

Radiological information regarding CSI was available in 470 adenomas. In 228 adenomas, CSI was not detected. In 63 adenomas, CSI was detected but Knosp grades 1–4 were not ascertained. In the remaining 179 adenomas, the

Table 1 Patient demographic, radiologic and histologic characteristics

	Functional adenomas (N = 141)				Nonfunctional adenomas (NFA, N = 360)		Total (501)
	PRL (53)	GH (44)	ACTH (42)	TSH (2)	ACTH negative (319)	Silent ACTH (41)	
Age (years), mean ± SD	34.1 ± 12.6	45.8 ± 12.6	44.7 ± 11.7	49.4, 46.9	57.6 ± 13.2	52.6 ± 14.7	52.5 ± 15.2
Men, N (%)	22 (41.5%)	23 (52.3%)	9 (21.4%)	2 (100%)	162 (50.8%)	14 (34.1%)	232 (46.3%)
Diameter (cm), mean ± SD	1.9 ± 1.1	2.1 ± 1.4	1.0 ± 0.7	1.1 ± 0.1	2.6 ± 1.0	2.3 ± 0.7	2.3 ± 1.1
Microadenomas, N (%)	9 (17.0%)	5 (11.4%)	27 (64.3%)	0 (0%)	4 (1.3%)	1 (2.4%)	46 (9.2%)
CSI, N (%)	22 (41.5%)	27 (61.4%)	7 (16.7%)	0 (0%)	164 (51.4%)	22 (53.7%)	242 (48.3%)
Ki-67 > 3%, N (%)	23 (43.4%)	7 (15.9%)	13 (31.0%)	0 (0%)	48 (15.0%)	6 (14.6%)	97 (19.4%)
Increased mitoses, N (%)	11 (20.8%)	3 (6.8%)	5 (11.9%)	0 (0%)	28 (8.8%)	2 (4.9%)	49 (9.8%)
P53 immunopositivity, N (%)	31 (58.5%)	29 (65.9%)	18 (42.9%)	0 (0%)	170 (53.3%)	17 (41.5%)	265 (52.9%)

Among the 179 patients with Knosp stages available, 115 had Knosp grade 3 and 4: 9 PRL, 15 GH, 6 ACTH, 0 TSH, 72 Non-ACTH NFA, 13 Silent ACTH

CSI cavernous sinus invasion

distribution of Knosp grades was as follows: 5 with Knosp 1, 59 with Knosp 2, 62 with Knosp 3, and 53 with Knosp 4. The group of patients with Knosp grades 3–4 included 47.0% men and had a mean age of 54.9 ± 13.8 years and a mean tumor diameter of 2.9 ± 1.1 cm. In this group, 21.7% adenomas had a high ki-67 index, 11.3% increased mitoses, and 57.4% p53 immunopositivity.

The median follow-up was 1.7 (IQR: 0.4–3.1) years. Short- and long-term clinical outcomes are shown in Table 2. Among functional PAs, prolactinomas were least likely to achieve biochemical remission (56.25%) or complete resection by MRI (54.7%) and had the highest rates of biochemical (11.3%) and tumor recurrence (5.7%).

Eighteen patients (3.6%) required multiple surgeries and radiation: 1 man with acromegaly (ACM), 2 women with Cushing's (CD), 9 (4 men and 5 women) with ACTH-negative NFA, and 6 (1 man and 5 women) with SCA. The mean age of this group was 46.1 ± 15.2 years, mean tumor diameter 3.0 ± 1.1 cm, and 72.2% of these PAs had CSI. High ki-67 was found in 33.3%, increased mitoses in 11.1%, and p53 immunopositivity in 55.6%.

In the entire cohort (501), all-cause mortality was 2.8% (14 of 501). Mean age at death was 65.7 ± 16.7 years.

Table 2 Postoperative short- and long-term outcomes

	Functional adenomas (141)				Nonfunctional adenomas (NFA, 360)		Total (501)
	PRL (53)	GH (44)	ACTH (42)	TSH (2)	ACTH negative (319)	Silent ACTH (41)	
Short-term outcomes							
Biochemical remission, N (%) for:	27 (56.25%)	26 (63.4%)	37 (88.1%)	2 (100%)	N/A	N/A	92 (69.2%)
Macroadenomas	19 (47.5%)	23 (62.2%)	12 (80%)	2 (100%)			
Microadenomas	8 (100%)	3 (75%)	25 (92.6%)	N/A			
Adenomas with CSI	8 (40%)	14 (56%)	3 (42.8%)	N/A			
Adenomas without CSI	19 (70.4%)	12 (75%)	27 (96.4%)	2 (100%)			
No tumor residual	29 (65.9%)	31 (72.1%)	36 (90%)	2 (100%)	222 (78.7%)	25 (67.6%)	345 (78%)
Long-term outcomes							
Biochemical recurrence, N (%)	6 (11.3%)	1 (2.3%)	3 (7.1%)	0 (0%)	N/A	N/A	10 (7%)
Tumor recurrence, N (%)	3 (5.7%)	1 (2.3%)	2 (4.8%)	0 (0%)	5 (1.6%)	2 (4.9%)	12 (2.6%)
Reoperation, N (%)	1 (1.9%)	1 (2.3%)	1 (2.4)	0 (0%)	0 (0%)	3 (7.3%)	6 (1.2%)
Radiation, N (%)	3 (5.7%)	4 (9.1%)	4 (9.5%)	0 (0%)	29 (9.1%)	11 (26.8%)	51 (10.2%)

"Biochemical remission" and "no tumor residual" are calculated for patients who had this information available at 3 months postoperatively. Five patients with prolactinoma and 3 patients with functional GH tumors did not have information regarding remission available. Nine patients with prolactinoma, 2 patients with functional ACTH tumors, one with functional GH tumor, 31 with ACTH-negative NFA and 4 with SCA did not have information regarding residual tumor at 3 months available

Statistical modeling for surgical outcomes and events prediction

The following parameters were evaluated in relation to event-free long-term survival: age, gender, diagnostic category, CSI (and Knosp grades 3–4 when available), tumor diameter, ki-67 > 3%, mitotic activity, and p53. Of these, CSI, Knosp grades 3–4, larger tumor diameter, and high ki-67 were identified as predictors of event-free long-term survival. The optimal cutoff point for tumor diameter to predict outcomes was 2.9 cm (sensitivity 51.4%, specificity 80.1%). Table 3 summarizes distribution of PAs across diagnostic categories and predictive models.

Best prediction of long-term course was achieved by Model 1 (M1) which included three parameters: tumor diameter ≥ 2.9 cm, CSI, and ki67 > 3% (Fig. 1). Contribution of each parameter to the model (hazard ratio) was as follows: 3.0 for tumor diameter, 2.7 for CSI, and 2.7 for ki-67 (p values < 0.01 for all). Model 1 also predicted short-term outcomes with a high specificity of 98.7%, and an odds ratio of 8.6 (95% CI 3.0–24.7) (Table 4). The group of patients with tumors that met all three characteristics: diameter ≥ 2.9 cm, CSI and ki67 > 3% (M1-positive) had a mean age of 51.4 ± 16.3 years, 61.1% male preponderance and mean tumor diameter of 3.8 ± 0.5 cm. The M1-positive group represented 3.6% of operated patients (Table 3) and had the highest representation within the prolactinoma group (5.7%) followed by ACTH-negative NFA (4.1%). The mean ki-67 labeling index of with M1-positive PA was 7.0% ± 5.3%. Mean follow-up time was 2.1 ± 1.2 years for M1-positive and 2.0 ± 1.6 for M1-negative patients. When Knosp grades 3–4 were considered instead of CSI (407 patients), the model's predictive ability was further strengthened (AIC 594 vs. 718). The contribution (hazard ratio) of each parameter to this model was as follows: 3.0 for Knosp grade, 3.0 for ki67 and 2.9 for tumor diameter (p < 0.01).

To better identify smaller functional adenomas at risk for persistent biochemical syndrome postoperatively, we assessed the performance of model 2 which included only two parameters: CSI and high ki-67. Almost 10% of tumors met both criteria, with highest PA type representation in the prolactinoma (20.8%) followed by GH-secreting adenoma (11.4%) groups (Table 3). The M2-positive group had a mean age of 45.5 ± 15.2 years, which was younger than the M2-negative group (53.3 ± 15.0; p < 0.01). The M2-positive group had 46% men and a mean tumor diameter of 2.7 ± 1.1 cm. The mean ki-67 in M2-positive group was 6.5% ± 4.3%. M2 had a specificity for short-term events of 93.1% and an OR of 3.3 (95% CI 1.8–6.0) (Table 4). Kaplan–Meier event-free survival analyses showed significant differences in long-term outcomes between the M2 groups (p < 0.01) (Fig. 2). The M2-positive group included 8 prolactinomas, 4 functional GH, 3 functional ACTH, 14 ACTH-negative NFA and 1 SCA that were not captured by model 1. Among the 8 prolactinomas, only 3 achieved biochemical remission at 3 months postoperatively. Mean follow-up time was 1.7 ± 1.3 years for M2-positive and 2.0 ± 1.6 for M2-negative patients.

Finally, we evaluated the model of the former "atypical adenoma" (WHO 2004 classification). Model 3 included ki67 > 3%, increased mitoses, and positive p53 immunohistochemistry. Five percent of tumors met criteria for this model, with the highest representation within prolactinoma (11.3%) followed by functional ACTH-adenoma (7.1%) groups (Table 3). The "atypical" group had a mean age of 45.7 ± 12.0 years (younger than "non-atypical" group: 52.9 ± 15.3 years; p < 0.05), 61.5% male preponderance, mean tumor diameter of 2.4 ± 1.2 cm, and 53.8% proportion of tumors with CSI. The mean ki-67 of patients with "atypical adenomas" was 7.5% ± 5.0%. There was no difference regarding gender or tumor diameter in the "atypical versus "non-atypical" groups. The M3-positive ("atypical") group had less favorable short-term outcomes than "non-atypical" (OR 2.3, 95% CI

Table 3 Distribution of adenomas across diagnostic categories and predictive models

Model	Functional adenomas (N = 141)				Nonfunctional adenomas (NFA, N = 360)		Total (501)
	PRL (53)	GH (44)	ACTH (42)	TSH (2)	ACTH negative (319)	SCA (41)	
M1-Positive Ki-67/diameter/CSI	3 (5.7%)	1 (2.3%)	0 (0%)	0 (0%)	13 (4.1%)	1 (2.4%)	18 (3.6%)
M2-Positive Ki-67/CSI	11 (20.8%)	5 (11.4%)	3 (7.1%)	0 (0%)	27 (8.5%)	2 (4.9%)	48 (9.6%)
M3-Positive Ki-67/p53/mitoses	6 (11.3%)	1 (2.3%)	3 (7.1%)	0 (0%)	15 (4.7%)	1 (2.4%)	26 (5.2%)

Adenomas positive for model 1 had ki-67 > 3%, positive p53 and increased mitotic activity. Adenomas positive for model 2 had ki-67 > 3%, and cavernous sinus invasion. Adenomas positive for model 3 had ki-67 > 3%, diameter ≥ 2.9 cm and cavernous sinus invasion

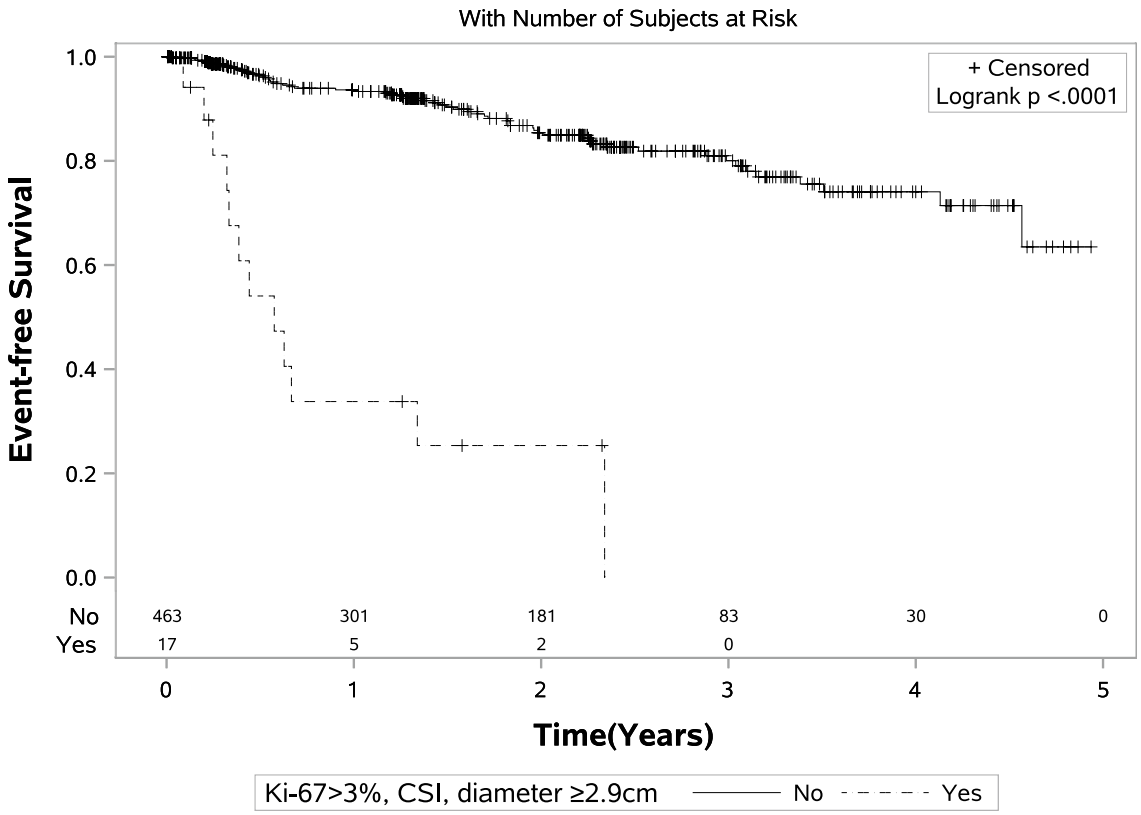

Fig. 1 Event-free survival Kaplan–Meier curve for two groups defined by cavernous sinus invasion, Ki-67, and diameter (Model 1). Events and outcomes considered were: biochemical recurrence, tumor recurrence, radiation therapy, and reoperation. Model 1 positive group is defined based on three parameters: cavernous sinus invasion, Ki-67 index > 3%, and diameter ≥ 2.9 cm. Number of patients at risks is shown above X-axis in each subgroup

Table 4 Model prediction for short-term outcomes

Model	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Odds ratio [95% CI]
M1 Ki-67/diameter/CSI	10.4	98.7	72.2	76.8	8.6 [3.0–24.7]
M2 Ki-67/CSI	19.5	93.1	50.0	76.5	3.3 [1.8–6.0]
M3 Ki-67/p53/mitoses	8.8	96.0	42.3	76.0	2.3 [1.0–5.2]

Short-term outcomes at 3 months postoperatively consisted in persistent biochemical activity for functional PAs and tumor residual for NFA

1.0–5.2), but with lower PPV than models 1 and 2 (Table 4). The Kaplan–Meier event-free survival analysis showed significant differences between M3 groups ($p < 0.05$) (Fig. 3). Mean follow-up time was 1.8 ± 1.3 years for M3-positive and 2.0 ± 1.6 years for M3-negative patients.

WHO 2017 special pituitary adenoma subtype analysis

Among the 501 patients, 22 were men with lactotroph adenomas, 19 had sparsely granulated somatotroph adenomas,

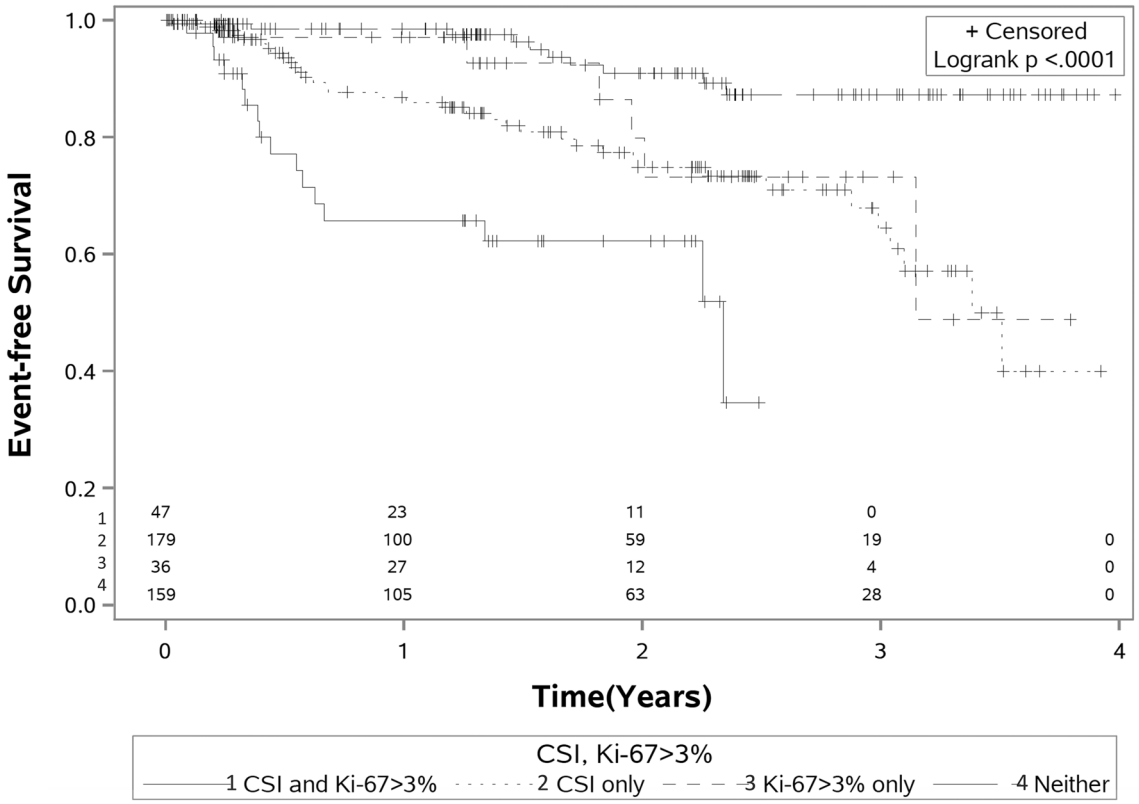

Fig. 2 Event-free survival Kaplan–Meier curve for groups defined by cavernous sinus invasion and Ki-67 (Model 2). Events and outcomes considered were: biochemical recurrence, tumor recurrence, radiation therapy, and reoperation. Graph represents groups of patients in four categories separated by ki-67 index and CSI characteristics. Number of patients at risks is shown above X-axis in each subgroup

41 SCAs (Table 5) and one Crooke cell adenoma.

Men represented 41.5% of patients with functional lactotroph adenomas and had a mean age at surgery of 38.6 ± 15.5 years and a prevalence of CSI of 45.5%. Histologically, 45.5% of lactotroph adenomas in men had high Ki-67 which was similar with lactotroph adenomas in women. Men with prolactinoma had larger tumors than women (2.6 ± 1.1 cm vs. 1.4 ± 0.9 cm; $p < 0.01$) and higher median preoperative prolactin (1162 ng/ml, IQR: 303–2237 vs. 171 ng/ml, IQR: 116.5–435, $p < 0.01$). Men were 5.0 times less likely than women to achieve normalization of prolactin levels at 3 months postoperatively (CI 1.5–16.4). Kaplan–Meier analyses did not find differences regarding long-term course between the genders ($p = 0.39$). Biochemical recurrence occurred in one man and five women, radiation in two men and one woman, and reoperation in none of the men and one woman.

SCA group represented 11.4% of NFA and had similar age at surgery, tumor diameter and proportion of tumors with CSI compared with ACTH-negative NFA. SCA patients were 2.9 times more likely to have tumor residual (CI 1.4–6.1) and had shorter long-term event-free survival than those with ACTH-negative NFA ($p < 0.01$) (Fig. 4). SCA group had the highest percentage of patients who required at least two surgeries and radiation during follow-up.

SG somatotroph adenomas group represented 44% of ACM group and had similar age, tumor diameter and proportion of tumors with CSI as those with DG somatotroph adenomas. Also, short- and long-term outcomes/events were similar.

During the study period, there were no cases of pituitary carcinomas.

Discussion

Key results: primary aims

Our large monocentric study evaluated the impact of demographic, clinical, radiological and histological parameters on the postoperative course. We identified two prognostic models: M1 (tumor diameter ≥ 2.9 cm, CSI, and ki-67 > 3%) suitable for macroadenomas, and M2 (CSI and ki-67 > 3%) that better identified at-risk smaller PAs. Other parameters including age, gender, diagnostic category, p53 and mitoses were not found significant in the proportional hazards model.

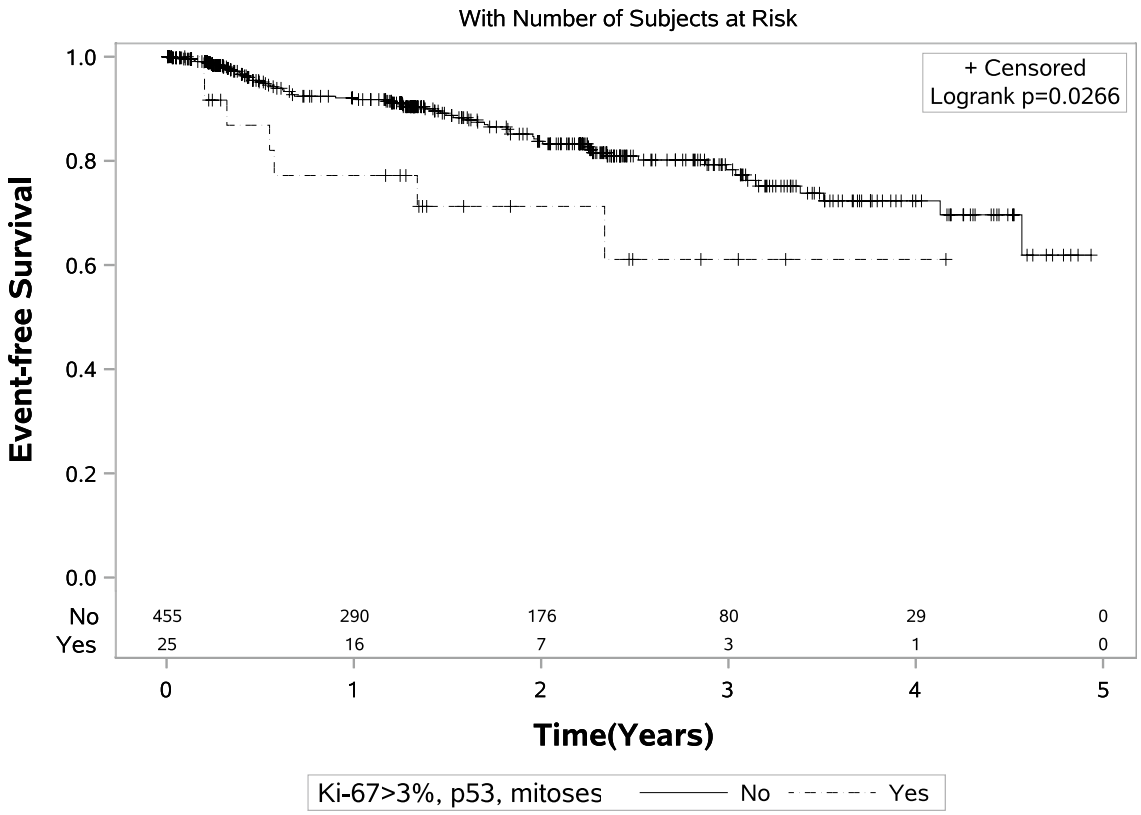

Fig. 3 Event-free survival Kaplan–Meier curve for WHO 2004 adenoma classification groups (Model 3). Events and outcomes considered were: biochemical recurrence, tumor recurrence, radiation therapy, and reoperation. Model 3 groups are defined based on three parameters: Ki-67 index > 3%, increased mitoses and p53 immunopositivity. Number of patients at risks is shown above X-axis in each subgroup

Table 5 Characteristics of special pituitary adenoma subtypes (WHO 2017)

	Lactotroph adenomas in men (N = 22)	Sparsely granulated somatotroph adenoma (N = 19)	Silent ACTH (N = 41)
Age (years) mean ± SD	38.6 ± 15.5	42.1 ± 11.3	52.6 ± 14.7
Men, N (%)	22 (100%)	8 (42.1%)	14 (34.1%)
Diameter (cm) mean ± SD	2.6 ± 1.1	2.5 ± 1.3	2.3 ± 0.7
CSI, N (%)	10 (45.5%)	16 (84.2%)	22 (53.7%)
Ki-67 > 3%, N (%)	10 (45.5%)	7 (36.8%)	6 (14.6%)
Increased Mitoses, N (%)	5 (22.7%)	1 (5.3%)	2 (4.9%)
P53 immunopositivity, N (%)	13 (59.1%)	15 (78.9%)	17 (41.5%)

CSI cavernous sinus invasion

Our study has practical implications for early risk stratification of operated patients, especially since grading systems for PA have evolved. The first grading system was proposed by Hardy in 1969 and took into consideration the erosion of the skull base and extrasellar structures [18]. After MRI became available, Knosp et al. proposed a grading system based on CSI [16]. In 2004, the WHO histologically defined two categories of PAs: "atypical" (i.e. three proliferative markers positive) and "non-atypical". However, outcome studies yielded discordant results regarding the prognostic value of the "atypical" tumors classification [9–14, 19–21]. Of note, the WHO definitions of the increased mitotic activity and p53 immunopositivity were qualitative and open to interpretation. In 2017, the WHO abandoned the term "atypical" PA, no longer recommended p53 routine evaluation, and emphasized the consideration of CSI as a marker of potential tumor aggressiveness. Nevertheless, a specific classification was not issued [15]. In 2013, Trouillas et al.

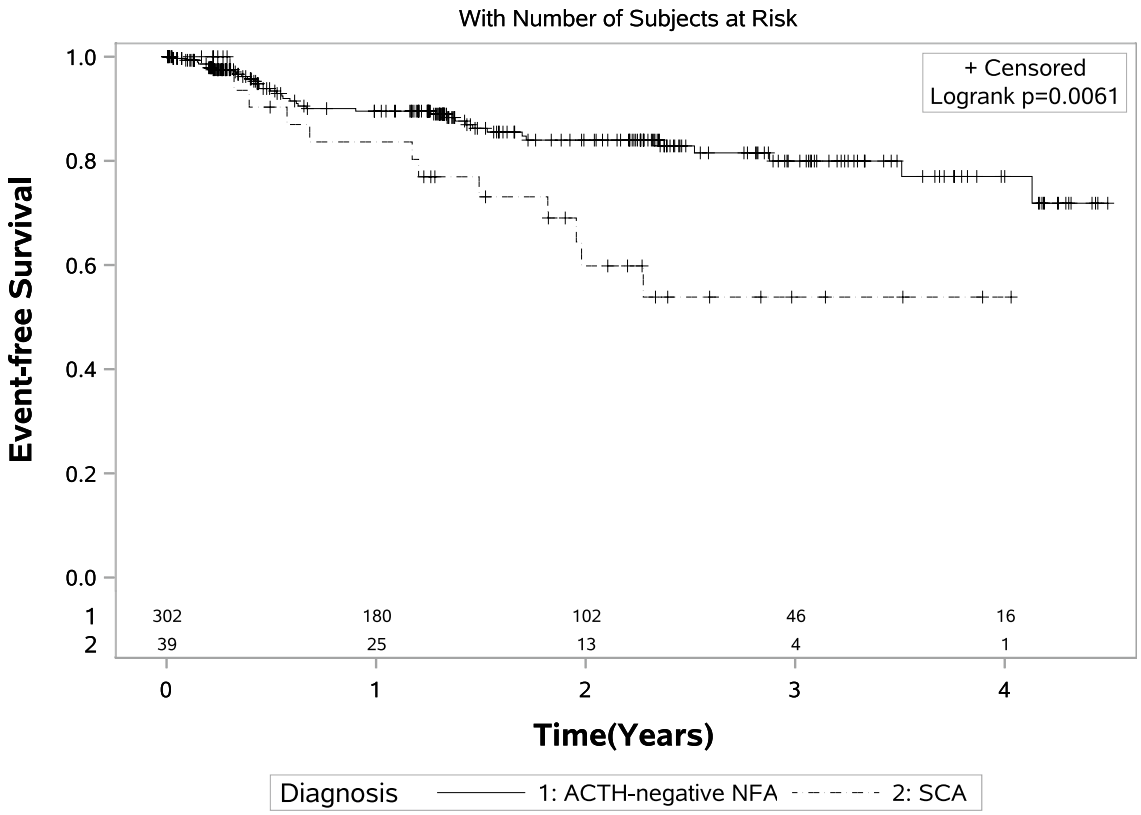

Fig. 4 Event-free survival Kaplan–Meier curve for two groups defined by ACTH immunopositivity in the nonfunctioning adenoma patients. Events and outcomes considered were tumor recurrence, radiation therapy, and reoperation. Number of patients at risks is shown above X-axis in each subgroup

proposed a five-tiered clinical-pathological classification and concluded that tumors invasive to the cavernous or sphenoid sinus with two out of three positive proliferation markers were more likely to have persistent or recurrent disease [22]. The use of such grading system is hampered by the lack of (a) a standardized terminology for histologic evaluation of PAs across centers and (b) an objective, verifiable evaluation of the sphenoid sinus invasion.

Several caveats apply to the histologic and radiologic markers used in current prognostication systems. The first applies to CSI, which can be approximately determined with modern MRI protocols [23]. To make CSI practical in risk stratification, inclusion of Knosp grade on radiology reports would be useful. The second caveat applies to the maximal tumor diameter: large tumors are usually incompletely resected [24], but tumor location, configuration and relation to surrounding structures are more relevant than maximal tumor diameter and not systematically considered in current analyses. Furthermore, functional adenomas are not defined by maximal tumor diameter but by biochemical activity which derives from pathophysiology rather than size. The third caveat applies to the ki-67 proliferation labelling index. Although high ki-67 has been associated with persistent disease or recurrence in many studies [25–33], the threshold is subject of debate. The 3% threshold was initially proposed by Thapar et al. based on ki-67 correlation with tumor invasiveness [34] and has been supported by WHO. Some studies endorsed lower ki-67 cutoffs (1.0–2.0%) [10, 35–37], while others did not [38]. While invasive PAs have been shown to have higher mean ki-67 than non-invasive ones [39, 40], tumor invasiveness and high ki-67 have independent contributions to long-term tumor outcomes. This is supported by our study (Fig. 2) and two other recent studies [22, 41]. Prolactinomas exhibit the highest proportion of tumors with high ki-67, but this marker may be influenced by preoperative medical treatment [39]. This aspect requires further study. Finally, ki-67 is a non-specific marker of tumor proliferation and further research is necessary to determine the predictive role of factors involved in pituitary tumorigenesis. For example, the expression of the pituitary tumor transforming gene (PTTG) [42, 43] was found higher in tumors with high ki-67 index [44] or invasive tumors [45].

Key results: secondary aims

The WHO 2017 classification indicated that certain PA subtypes are at higher risk for disease persistence or recurrence [46]. Our study confirmed that patients with SCA were 2.9 times more likely to have a tumor residual and had a lower event-free survival than ACTH-negative NFA. Cooper et al. found a median time to tumor recurrence of 3 versus 8 years for the two categories [47]. Other studies indicated that long-term recurrence-free survival was similar for SCA and ACTH-negative NFA treated with a similar adjuvant radiation protocol [48]. Per WHO 2017, another special PA subtype is lactotroph adenomas in men. In our study, men were 5 times less likely than women to have normal prolactin levels at 3 months postoperatively which is consistent with other studies [49]. However, the long-term event-free survival in our patients with prolactinoma was similar across genders. Sparsely granulated (SG) somatotroph adenomas are another special WHO subtype. Previous studies yielded controversial results: some supported lower rates of remission in patients with SG GH-secreting adenomas [50], and others did not [51]. We could not demonstrate lower remission rates or event-free survival in SG tumors compared with DG. This may be explained by the relatively small number of patients in each category. Finally, the WHO suggested Crooke cell adenomas may have a more aggressive course as suggested by case reports [52]. We only had one patient with this rare type of PA.

Limitations

Our study has several limitations. The design was a retrospective database query, similar to most previous studies. The median follow-up was less than 2 years, which is a relatively short period. However, our models had excellent specificity in identifying patients at risk at 3 months postoperatively for both functional and NFA. While the definition of a clinically aggressive PA remains controversial [7, 9, 22, 53–55], several studies indicate that these tumors tend to grow rapidly and remain biochemically active or cause early biochemical recurrence [6, 56, 57]. Finally, the number of patients with acromegaly, Cushing's disease and prolactinoma caused by invasive tumors with high proliferation markers was small, which means the long-term follow-up prediction of the models are driven by the NFA group.

Conclusion

Systematic consideration of tumor size, tumor invasion, ki-67 index and certain histologic subtypes predict postoperative outcomes in a subset of patients with PAs. These patients benefit from close surveillance and multidisciplinary management. Nevertheless, many PAs at risk for tumor progression and recurrence are not identified by this approach. The updated more rigorous classification of PAs recommends evaluation of cell-specific hormone transcription factors. This may be helpful to determine new prediction criteria, especially for NFA, which have heterogenous biology and variable clinical behaviors. In addition, research directed at molecular profiling of PAs is necessary to determine the drivers of tumor growth and invasion.

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Compliance with ethical standards

Conflict of interest The authors have nothing to disclose.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of Emory's institutional and/or national research committee.

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