Real-life clinical impact of a five-tiered classification of pituitary tumors

Nicolas Sahakian, Romain Appay, Noémie Resseguier, Thomas Graillon, Cécilia Piazzola, Cécilia Laure, Dominique Figarella-Branger, Jean Régis, Frédéric Castinetti, Thierry Brue, et al.

Real-life clinical impact of a five-tiered classification of pituitary tumors

Nicolas Sahakian[1], Romain Appay[2], Noémie Resseguier[3,4], Thomas Graillon[5], Cécilia Piazzola[1], Cécilia Laure[6], Dominique Figarella-Branger[2], Jean Régis[7], Frédéric Castinetti[1], Thierry Brue[1], Henry Dufour[5,*] and **Thomas Cuny[1,*]**

[1]Aix Marseille Univ, MMG, INSERM U1251, MARMARA Institute, APHM, Hôpital de la Conception, Department of Endocrinology, CRMR HYPO, Marseille, France, [2]APHM, CHU Timone, Service d'Anatomie Pathologique et de Neuropathologie, Marseille, France, [3]Aix Marseille Univ, APHM, Hôpital de la Timone, Service d'Epidemiologie et d'Economie de la Santé, Unité de Recherche Clinique, Direction de la Recherche en Santé, Marseille, France, [4]EA3279, CEReSS, Health Service Research and Quality of Life Center, Aix-Marseille University, Marseille, France, [5]Aix Marseille University, MMG, INSERM U1251, MARMARA Institute, APHM, Hôpital de la Timone Department of Neurosurgery, CRMR HYPO, Marseille, France, [6]Department of Endocrinology, University Hospital of Montpellier, France, and [7]Aix Marseille University, INS-UMR1106, Department of Functional and Stereotactic Neurosurgery, CHU Timone, Marseille, France

[*](H Dufour and T Cuny contributed equally to this work)

Correspondence should be addressed to T Cuny
Email
thomas.cuny@ap-hm.fr

Abstract

Introduction: Usually benign, pituitary tumors (PT) can be invasive and aggressive with a propensity to progress and/or recur. Trouillas's clinicopathological classification attempts to predict the evolutionary risk of a PT. In this study, we assessed the prognostic value of this classification in an independent patient cohort and analyzed its impact on treatment strategies.
Patients and methods: In this study, 607 patients operated on between 2008 and 2018 for a PT were included. Grading was established based on invasion, proliferative activity (Ki-67, mitotic index) and p53 positivity. The therapeutic management following surgery was analyzed. Progression-free survival (PFS) of the graded tumors was estimated (Kaplan–Meier method and log-rank test) and a multivariate analysis was performed (Cox regression model).
Results: Grading identified non-invasive PT without (grade 1a: 303 cases) or with proliferative activity (grade 1b: 53 cases) and invasive PT without (grade 2a: 202 cases) or with proliferative activity (grade 2b: 49 cases). The mean follow-up was 47 ± 30 months (median: 38 months). Progression/recurrence occurred in 127 cases. Grades were significant and independent predictors of PFS ($P < 0.001$) with a 4.8-fold higher risk of progression/recurrence in grade 2b as compared to grade 1a. As second-line therapy, gamma knife or conventional radiotherapy controlled tumor growth in 91.6 and 100% of cases, respectively, irrespective of the grade. Proliferative tumors exposed the patient to a 9.5-fold higher risk of having ≥3 adjuvant therapeutic lines as compared to non-proliferative tumors.
Discussion: Grading of a PT according to Trouillas's classification predicts its risk of progression and should advocate for a personalized therapeutic approach in invasive and proliferative tumors.

Significance statement

This is the first study to assess, on a cohort of 607 well-characterized patients, the real-life therapeutic impact of the five-tiered clinicopathological classification of pituitary tumors. First, we validate that pituitary tumor grades predict the evolutionary risk of the tumor, with a significant higher risk of progression/recurrence in invasive and/or proliferative tumors (mean follow-up: 47 ± 30 months, median: 38 months). Moreover, our study provides evidence that patients with proliferative tumors have a higher risk to be retreated after primary surgery and point toward the fact that radiotherapy can successfully control tumor growth in case of progression or recurrence. Our findings advocate for a personalized therapeutic approach in clinically aggressive pituitary tumors.

Introduction

Pituitary adenomas, referred to as pituitary neuroendocrine tumors (PitNET) or, simply, pituitary tumors (PT) account for 10–15% of all intracranial neoplasms (1, 2, 3). Usually benign, nearly 40% of PT show, at diagnosis, features of invasiveness in the cavernous and/or the sphenoidal sinus (2, 4, 5). Moreover, a subset of PT will be characterized by aggressive behavior, defined as a rapid growth potential, resistance to the conventional treatment or even multiple recurrence following initial surgery (6, 7). In exceptional cases, distant metastasis may develop, a condition known as pituitary carcinoma (7). In 2004, the World Health Organization introduced the notion of atypical adenoma, which emphasized their propensity to exhibit an unusual behavior associated with an uncertain evolutionary risk (8). Atypical adenomas were characterized by at least two of these three following criteria: Ki-67 labeling index ≥3%, extensive nuclear p53 immunostaining and high mitotic index, such as their reported incidence ranged from 3 to 19% (9, 10). In 2013, Trouillas and coworkers proposed a clinicopathological classification, which relied on the combined analysis of invasion of the tumor, proliferation (Ki-67, mitotic count) and nuclear p53 expression, achieved to stratify the evolutionary risk of a PT according to five distinct grades (11). While its prognostic value was subsequently confirmed in other independent studies, the impact of the tumor grading on the therapeutic management of the patient was never addressed so far.

Therefore, the aim of this study was to validate the prognostic value of the five-tiered classification in an independent monocentric cohort of 607 patients operated for a PT and furthermore, to focus on the therapeutic outcomes ensuing from this classification.

Patients and Methods

Study design and patients

We conducted an observational retrospective study at the university hospital of Marseille, French national reference center of rare pituitary diseases. A total of 1164 patients who were referred to our center for a pituitary lesion diagnosed between January 2008 and December 2018 were reviewed. The endocrine and neuroradiological characteristics were documented for every patient before any treatment. All of them underwent a 1.5T pituitary MRI before surgery. PT were classified as microadenomas (tumor diameter <10 mm) or macroadenomas (tumor diameter ≥10 mm) (12). Radiological invasiveness was defined according to the Knosp system in which grades 3 and 4 defined tumors invading the cavernous sinus (4). All patients underwent endoscopic transsphenoidal surgery performed by expert neurosurgeons (HD and TG). The degree of surgical removal was defined based on the surgeon report and classified as follows: growth total resection (GTR) when the removal was complete and subtotal resection (STR) when the tumor removal was incomplete.

After surgery, patients were followed in the department of endocrinology with a measurement of hormonal level and a pituitary MRI at 3 and 12 months and at least annually until the last follow-up (LFU). The present study was approved by the research council of Aix-Marseille University (PADS authorization number: 2019-152). This retrospective monocentric observational design did not require a written consent by the patients according to the French legislation.

Tumoral classification of PT

For each tumor, proliferation markers were analyzed according to previously described procedures (11). All PT samples were fixed in formalin. The mitotic count was calculated after hematoxylin-eosin staining and expressed as an absolute number/ten high power fields (HPF). The Ki-67 labeling index was evaluated as a percentage of positive nuclei. The expression of p53 was considered positive if > 10 strongly positive nuclei per 10 HPF were recorded (11). Proliferative tumors were defined by the presence of at least two of the following criteria (11): Ki-67 ≥ 3%, mitotic count > 2/10 HPF and p53 positivity. Invasion of the cavernous sinus and/or dura matter was histologically investigated as well; invasion was retained only if pituitary tumor tissue was found within dura mater, bone and/or respiratory mucosa. PT were classified as grade 1a (non-invasive and non-proliferative tumor), grade 1b (non-invasive and proliferative), grade 2a (invasive and non-proliferative), grade 2b (invasive and proliferative) and grade 3 (pituitary carcinoma) (Supplementary Table 1, see section on supplementary materials given at the end of this article). The grade of the tumor was established based on the first surgery only. Pituitary tumor subtyping by immunohistochemistry was performed according to criteria incorporated in the WHO classification available for the period of analysis. Tumors that were negative for all pituitary antibodies were considered immunonegative because we did not routinely perform immunostaining for transcription

factors. Thus, we did not classify any tumor as null cell PT in our study.

Criteria of remission and progression

The primary endpoint in this study was the time between the first surgery and the progression/recurrence or the LFU of a controlled disease.

Patients were in complete remission after surgery if they had no evidence of pituitary disease at the biological and radiological levels. In secreting tumors, patients were considered controlled if they had normal hormone levels with an anti-secretory drug and a stable or non-visualized remnant on MRI; in non-secreting tumors, patients were considered controlled if they had a stable remnant. While these two populations of patients were pooled for the statistical analysis, we compared them in a dedicated table (Supplementary Table 2).

Recurrence was diagnosed in patients with the appearance of a previously unknown PT residue and/or if an increase in the pituitary hormone levels occurred during the follow-up of the patient. Progression was defined by the growth of a known tumor residue. In most cases, progression or recurrence led to discussion of a new therapeutic adjustment. Patients with recurrence, progression or modification of their treatment were pooled for the statistical analysis.

Remission/control or progression/recurrence status was defined for all patients during a multidisciplinary team committee. An informed consent for the therapeutic decision was obtained from each patient after full explanation of its purpose. Therapeutic interventions were validated during a multidisciplinary team committee in a personalized approach which took into account the patient condition, the size and location of the tumor remnant, the existence of one or several pituitary deficiencies and/or the existence of a hormonal hypersecretion. Gamma knife radiosurgery (GKRS) was preferably used in patients presenting a relatively low target volume, well delineated on MRI, sufficiently far (i.e. > 5 mm) from the optic chiasm (to avoid any risk of induced optic neuritis) and not previously treated by any radiation technique (which would increase the rate of adverse effects and/or need to decrease the dose, thus decreasing the likelihood of remission) (13). On the contrary, conventional radiotherapy was preferred in case of big or multiple target volume with pre-existing hypopituitarism.

A wait-and-see attitude could have been retained in certain patients with a minimal tumor progression without visual or neurologic consequences and hormonal burden or when the patient condition/choice did not permit any additional treatment.

Statistical analysis

Statistical analyses were performed using graphPad Prism software (*version 8.0.1*) and R software (http://www.R-project.org). Quantitative continuous variables were reported as median with IQR and mean ± S.D. Unpaired continuous variables were compared using a parametric Student's *t*-test. For multiple comparisons, we performed an ANOVA or a non-parametric Kruskal–Wallis test. Comparison of unpaired categorical variables was performed with a non-parametric Mann–Whitney test. Comparison of discrete variables was performed by chi-square test if applicable, otherwise with Fisher's test. In addition, progression-free survival for the different grades was estimated using the Kaplan–Meier method and the progression-free survival curves obtained for grades were compared using the log-rank test. Finally, factors predictive of recurrence or progression were determined by univariate and multivariate logistic regression. Firth correction was applied. Hazard ratios (HR) and their 95% CI were estimated for each factor. A value of $P<0.05$ was considered statistically significant. All tests were two-sided.

Results

Patients

Among the 1164 patients referred to our tertiary center during the study's period, 202 were not included, either because they did not undergo pituitary surgery and/or because the final diagnosis was not a primitive PT (i.e. tumors that does not develop from the anterior pituitary cells like metastasis or pituitary sarcoidosis). In the remaining 962 patients, 355 were excluded for one of the following exclusion criteria: patients with pituitary apoplexy ($n=29$) or genetically related PT ($n=20$), patients with a follow-up of less than 12 months ($n=218$) and those for whom the histopathological analysis was not contributive ($n=88$). We decided to rule out patients with genetically related PT because they represent a specific subset of patients who are usually younger at diagnosis and with more aggressive phenotypes of PT.

Following these criteria, we included 607 patients (Fig. 1). Among 607 patients, there were 316 (52%) women. The mean age at diagnosis was 52 ± 15 years (14–87 years) with the mean follow-up of 47 ± 30 months

(12–146 months, median: 38 months, IQR: 23–66 months). Three-hundred twenty (52.7%) patients had a non-secreting PT, 154 (25.4%) had acromegaly, 96 (15.8%) had a Cushing's disease, 27 (4.5%) had prolactinoma and eventually, 5 (1.6%) had either TSH-secreting PT or a co-secretion (Table 1). There were 199 patients who were treated preoperatively, most of them ($n=128$) with somatostatin receptor ligands (SRL) for an active acromegaly or dopamine agonist (DA) for a prolactinoma ($n=27$) (see details in Table 1). All patients with prolactinomas who underwent surgery were either intolerant to DA and/or the drug failed to control tumor growth and/or hypersecretion. There was a majority of macroadenomas ($n=498$, 82%) with a mean maximal tumor diameter of 23.5 ± 8.7 mm as compared to 5.7 ± 2.8 mm for microadenomas ($P < 0.0001$). Around 192 (38.5%) tumors were invasive according to the Knosp grading system. A total of 479 (78.9%) patients underwent a GTR and 128 (21.1%) had a STR. At 3 months postoperative, 155 patients had a PT residue and 55 had persistence of hormonal hypersecretion. Of note, 51 patients who benefited from GTR exhibited a PT residue on the 3-month MRI, which could be due to several reasons like unseen PT residue during surgery (because of excessive bleeding for instance) and false estimation of a millimetric PT residue at 3 months which ultimately turned out to be a post-surgical remodeling. At the LFU, 127 (21%) had either progression ($n=80$) or recurrence ($n=47$), 93 (15%) patients were controlled and 387 (64%) were in remission. Compared to patient in remission, patients with controlled disease were older (56 ± 16 vs 52 ± 14 years, $P=0.0243$), had a greater mean maximal tumor size at diagnosis (26.1 ± 9.9 mm vs 17.3 ± 9.6 mm, $P<0.0001$), a higher rate of cavernous invasion (49% vs 13%, $P<0.0001$) and a higher rate of subtotal surgical resection (55% vs 14%, $P<0.0001$). While proliferative markers were not different between the two groups, grade 1a (non-invasive) tumors were most frequent in the remission group and grade 2a (invasive) tumors were most frequent in the controlled group, respectively (Supplementary Table 2).

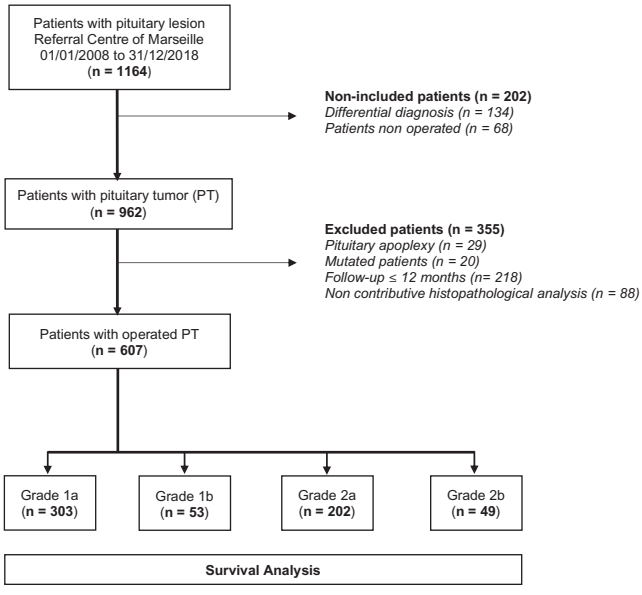


Figure 1
Flow chart of the study.

Tumors characteristics

The histopathological analysis identified 143 (23.5%) immunonegative tumors, 145 (23.9%) were positive for LH/FSH, 116 (19.1%) were positive for GH and the same proportion for ACTH, 47 (7.7%) were mixed GH/PRL, 27 (4.4%) were positive for PRL, 2 (<1%) for TSH and eventually, 11 (1.7%) were positive for multiple hormones (Table 1). There were eight patients with GH-positive tumor, however, without GH and IGF-1 excess, a condition defined as silent somatotroph tumor. Likewise, 19 patients who had no biochemical evidence of hypercortisolism while having tumor positivity for ACTH were considered as having a silent corticotroph tumor (Supplementary Table 3).

Our cohort identified that 303 (50%) were grade 1a, 53 (8.7%) were grade 1b, 202 (33.4%) were grade 2a and 49 (7.7%) were grade 2b tumors (Table 1). It has to be noted that 49 grade 2a tumors had a Knosp score < 3, meaning that invasion was diagnosed on histopathological and/or surgical ascertainment. At the time of pituitary surgery, there was no pituitary carcinoma (grade 3). Characteristics of the patients according to the PT grade are provided in Supplementary Table 4.

Progressive and recurrent pituitary tumors

Progression/recurrence occurred in 127 (21%) patients. In detail, it occurred in 20/303 (6.6%) grade 1a, 14/53 (26%) grade 1b, 62/202 (30.7%) grade 2a and 31/49 (63.2%) grade 2b tumors. Patients with progressive/recurrent tumors were younger as compared to their non-progressive counterparts (48 ± 13 yo vs 53 ± 15 yo, $P=0.0004$). The maximal diameter at diagnosis was significantly larger in progressive as compared to non-progressive tumors (25.3 ± 10.9 mm vs 18.9 ± 10.2 mm, $P<0.0001$, Table 2). There were 86 (67.7%) patients with radiologically invasive PT. Proliferative markers (Ki-67: 3 ± 3% vs 2 ± 2% and mitotic count: 1.3 ± 1.9

Table 1 Characteristics of the population of patients (n = 607). Size (in mm) was available for 460 tumors. Data are presented as mean ± S.D. or as n (%).

	All	Immunonegative	LH/FSH	GH	GH/PRL	PRL	ACTH	Other PT
Cases, *n*	607	143	145	116	47	27	116	13
Age at diagnosis	52 ± 15	58 ± 13	59 ± 11	47 ± 12	46 ± 13	36 ± 14	47 ± 15	44 ± 14
Follow-up	47 ± 30	48 ± 29	46 ± 32	48 ± 32	51 ± 27	42 ± 28	43 ± 28	61.8 ± 38.2
(IQR))	23–66	28–65	19–60	22–70	26–72	21–57	21–53	22–88
Secreting PT	287 (47%)	0	0	108 (93%)	47 (100%)	27 (100%)	97 (94%)	6 (46%)
PSMT	199 (33%)	10 (7%)	9 (6%)	92 (79%)	39 (83%)	27 (100%)	17 (15%)	6 (46%)
Radiological features								
Mean size	20 ± 10.6	26.7 ± 7.8	27.4 ± 8.5	16.7 ± 7	13.3 ± 7	16.6 ± 10.5	10 ± 7.8	22.4 ± 13.1
Microadenoma (<10 mm)	109 (18%)	0	2 (1%)	13 (11%)	13 (28%)	9 (33%)	70 (60%)	2 (15%)
Mean size	5.7 ± 2.8	–	6	7.5	7.2	7	4.7	7.5 ± 3.5
Macroadenoma (≥10 mm)	498 (82%)	143 (100%)	143 (99%)	103 (89%)	34 (72%)	18 (27%)	46 (40%)	11(85%)
Mean size	23.5 ± 8.7	26.7 ± 7.8	27.8	18.4	15.8	21.3	18.6	25.8 ± 12
Knosp ≥ 3	192 (32%)	66 (46%)	54 (37%)	40 (34%)	7 (15%)	6 (22%)	17 (15%)	2 (15%)
Surgical outcome								
GTR	479 (79%)	96 (67%)	108 (75%)	96 (83%)	41 (87%)	20 (74%)	108 (93%)	10 (77%)
STR	128 (21%)	47 (33%)	37 (25%)	20 (17%)	6 (13%)	7 (26%)	8 (7%)	3 (23%)
Histological features								
Ki67 (%)	2 ± 2	2 ± 3	2 ± 2	1 ± 1	2 ± 2	2 ± 3	2 ± 2	2 ± 3
Ki67 ≥ 3%	156 (26%)	47 (33%)	36 (25%)	21 (18%)	5 (11%)	9 (33%)	35 (30%)	3 (23%)
Mitosis	0.7 ± 1.4	0.8 ± 1.5	0.6 ± 1.2	0.6 ± 1.2	0.4 ± 0.9	0.8 ± 1.4	1 ± 1.8	0.7 ± 1.5
Mitosis > 2/10 HPF	128 (21%)	34 (23%)	27 (18%)	20 (17%)	6 (13%)	6 (22%)	33 (28%)	2 (15%)
p53 (positivity)	79/570 (14%)	22/128 (17%)	12/126 (9%)	18/104 (17%)	7/45 (15%)	6/25 (24%)	13/100 (13%)	1/12 (8%)
Histological invasion	101 (17%)	24 (17%)	18 (12%)	27 (23%)	8 (17%)	3 (11%)	18 (15%)	3 (23%)
Grade								
1a	303 (50%)	55 (38%)	72 (50%)	55 (47%)	28 (59%)	14 (52%)	72 (62%)	7 (54%)
1b	53 (9%)	14 (10%)	10 (7%)	7 (6%)	4 (8%)	4 (15%)	13 (11%)	1 (8%)
2a	202 (33%)	62 (43%)	53 (36%)	44 (38%)	13 (28%)	7 (26%)	20 (17%)	5 (38%)
2b	49 (8%)	12 (8%)	10 (7%)	10 (9%)	2 (4%)	2 (7%)	11 (9%)	0
Post operative status								
MRI residue	155 (25%)	54 (37.7%)	54 (37%)	20 (17%)	6 (13%)	6 (22%)	12 (10%)	3 (23%)
Biochemical persistence	55 (9%)	0	0	24 (21%)	10 (21%)	12 (44%)	9 (8%)	0
Last follow-up status								
Remission/controlled	480 (79%)	98 (68.5%)	119 (82%)	93 (80%)	44 (93%)	24 (89%)	89 (77%)	13 (100%)
Recurrence/progression	127 (21%)	45 (31%)	26 (18%)	23 (20%)	3 (7%)	3 (4%)	27 (23%)	0
Tumor progression	116 (19%)	45 (31%)	26 (18%)	14 (12%)	3 (7%)	3 (4%)	21 (18%)	0
Hypersecretion	42 (7%)	0	0	17 (15%)	3 (7%)	3 (4%)	17 (15%)	0

ACTH, adrenocorticotropic hormone; GH, growth hormone; GTR, gross total resection; PRL, prolactin; PSMT, pre-surgical medical treatment; STR, subtotal tumor resection; TSH, thyroid-stimulating hormone.

vs 0.6 ± 1.2, $P<0.001$ for both) and nuclear p53 expression (positivity in 25/98 (25%) vs 54/442 (12%), $P<0.001$) were significantly higher in progressive tumors as compared to non-progressive ones (Table 2). High proliferative markers according to the Trouillas classification (Ki-67% ≥ 3% and mitotic count > 2/10 HPF) were found in 42 and 35% of progressive PT, compared to 21% and 17% in non-progressive one, respectively ($P<0.0001$ for both).

The Kaplan–Meier progression-free survival curves obtained for different grades were statistically different ($P<0.001$, Fig. 2) irrespective of the secreting status of the tumors (P < 0.001 between grades, Supplementary Fig. 1). Moreover, we observed that the risk of progression/recurrence decreased by 17% with every achieved decade following the age of PT diagnosis in each patient ($P=0.007$). Finally, the existence of a radiological invasion ($P=0.002$) significantly increased this risk (Table 3). Grades 1b and 2b tumors were independently associated with a higher risk of recurrence/progression with respect to grade 1a tumors (HR: 3.27; 95% CI: 1.59–6.56 and HR: 4.8; 95% CI: 1.85–11.29, respectively). Two patients with grade 2b tumors developed distant metastasis (grade 3) during their follow-up and one of them passed away 144 months after the first surgery. On the contrary, invasion was not significantly associated with prognosis for grade 2a tumors (HR: 2.14; 95% CI: 0.83–4.98). Interestingly, we found that grade 2a PT with radiological invasion (Knosp ≥ 3) had a higher risk of progression/recurrence as compared to grade 2a PT without radiological invasion (Knosp < 3) ($P<0.05$, Supplementary Fig. 2). When assessing the tumor type, we found a lower risk of recurrence/progression for GH-positive and GH/PRL-positive tumors, while the comparison of secreting vs non-secreting PT according to the grades suggested a better prognosis of secreting PT.

Table 2 Comparison of patients with pituitary tumors in remission/controlled and progressive/recurrent diseases.

	Control/remission (n = 480)	**Progression/recurrence** (n = 127)	***P*-value**
Age at diagnosis (mean ± S.D.)	53 ± 15	48 ± 13	0.0004
Sex (female/male, %)	54/46	45/55	NS
Follow-up (month), mean ± S.D. (IQR)	47 ± 31 (23–64)	46 ± 28 (25–67)	NS
PSMT	170/480 (35%)	29/127 (22%)	0.0073
GH-secreting PT	108/130 (83%)	20/25 (80%)	NS
PRL-secreting PT	21/21 (100%)	2/2 (100%)	NS
ACTH-secreting PT	8/78 (10.2%)	4/18 (22.2%)	NS
Tumor size (mm)			
Mean size of the whole cohort	18.9 ± 10.2	25.3 ± 10.9	<0.0001
Microadenoma	98 (20%)	11 (9%)	
Mean size	5.6 ± 2.8	6.1 ± 3.1	
Macroadenoma	382 (80%)	116 (91%)	NS
Mean size	22.6 ± 8.3	27.2 ± 9.4	<0.0001
Radiological invasiveness (Knosp ≥ 3)	106 (22%)	86 (68%)	<0.0001
Surgical outcome			
GTR	413 (86%)	66 (52%)	<0.0001
STR	67 (14%)	61 (48%)	<0.0001
Histological subtype			NS
Immunonegative	98 (20%)	45 (35%)	
LH/FSH	119 (25%)	26 (20%)	
GH	93 (19%)	23 (18%)	
GH/PRL	44 (9%)	3 (2%)	
PRL	24 (5%)	3 (2%)	
ACTH	89(18%)	27 (21%)	
TSH	2 (<1%)	0	
Plurihormonal	11 (2%)	0	
Histological features			
Ki67(mean ± S.D.)	2 ± 2	3 ± 3	<0.0001
Ki67 ≥ 3%	102 (21%)	54 (42%)	<0.0001
Mitotic count (mean ± S.D.)	0.6 ± 1.2	1.3 ± 1.9	<0.0001
Mitoses > 2/10 HPF	83 (17%)	45 (35%)	<0.0001
P53 positivity	54/442 (12%)	25/98 (25%)	0.0008
Invasion	77 (16%)	24 (20%)	NS
Histological grade			
1a	283 (59%)	20 (16%)	<0.0001
1b	39 (8%)	14 (11%)	NS
2a	140 (29%)	62 (49%)	<0.0001
2b	18 (4%)	31 (24%)	<0.0001
PT residue (3 months postoperative)	75 (16%)	80 (63%)	<0.0001
Hormonal hypersecretion (3 months postoperative)	29 (6%)	26 (20%)	<0.0001

NS, non-significant; PSMT, pre-surgical medical treatment.

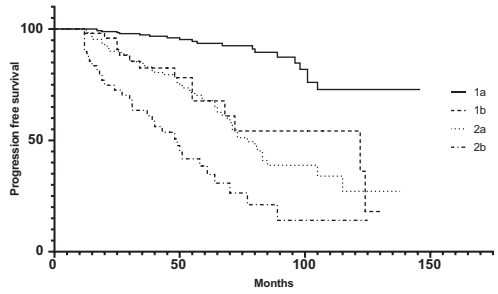
Therapeutic management of progressive/recurrent pituitary tumors

Among 127 patients with progressive tumors, 114 (89.7%) underwent an additional first-line adjuvant therapy, 17 (13.3%) a second-line therapy and eventually, 5 patients (3.9%) had ≥3 additional therapies (Fig. 3).

First-line adjuvant therapy

Adjuvant treatment after surgery concerned grade 1a (n=17/20), 1b (n=10/14), 2a (n=57/62) and 2b (n=30/31) progressive tumors (Fig. 3). The mean delay between surgery and this new therapeutic intervention was of 59.9 ± 31.7, 51.1 ± 35.4, 45.5 ± 25.5 and 35.3 ± 21.8 months for grades 1a, 1b, 2a and 2b, respectively, a difference that reached statistical significance between grades 1a and 2b (P=0.0181) but neither 2a (P=0.5634) nor 1b (P>0.99).

The treatment consisted of gamma knife radiosurgery (GKRS) in 60 cases (52%). In this population of patients, the mean follow-up was 49.4 ± 27.3 months (median: 49 months). The median marginal dose delivered to the residue was 18.9 ± 4.8 Gy. Progression/recurrence was controlled in 55 out of 60 (91.6%) patients including 17/18 (94.5%) with proliferative tumors (grade 1b (n=3) and



Number at risk

Months	12	24	36	48	60	72	84	96	108	120
1a	303	225	171	127	103	79	51	33	20	11
1b	53	41	26	19	13	9	7	7	5	3
2a	202	144	106	82	57	33	19	10	5	3
2b	49	34	26	18	10	5	4	1	1	1
total	607	444	329	247	183	126	81	51	31	18

Figure 2

Progression-free survival according to the histological grade of the pituitary tumor (1a; 1b; 2a and 2b) in patients with a follow-up ≥ 12 months.

grade 2b ($n=14$)) (Fig. 3). Conventional radiotherapy was performed in 11 (9.6%) patients, all of them having grade 2 tumors. The mean follow-up in this subset of patient was 38 ± 25.2 months (median: 30 months). The mean delivered dose was 50.2 ± 2.5 Gy (27.3 ± 1.7 sessions over 44.9 ± 5.7 days) and tumor growth was controlled in 100% of cases. Finally, 23 (20%) patients had a second surgery in a mean delay of 37.9 ± 30.3 months (12–105 months) with no further tumor progression/recurrence in 61% (9 patients required an additional second-line adjuvant therapy). Other therapies were proposed in 20 (17.5%) patients, including steroidogenesis inhibitors in 7 patients with uncontrolled Cushing's disease, SRL and/or dopamine agonist in 11 patients with acromegaly, dopamine agonist therapy in 2 additional patients, one with a prolactinoma and the other one with a non-secreting PT. Overall, the risk of having at least one more additional therapy after surgery with grade b tumors was 3.7-fold higher as compared to grade a tumors ($P=0.04$).

Second- or more line additional therapies

A total of 17 patients needed a second adjuvant therapy (grade 1a, $n=3$; grade 1b, $n=3$; grade 2a, $n=6$; grade 2b, $n=5$). GKRS was performed in five patients with control of tumor growth in four of them (80%), while conventional radiotherapy resulted in control of the disease in three out of four patients (75%). Finally, five patients underwent ≥3 additional therapies (Table 4), all of them carrying a proliferative pituitary tumor. As such, the risk of having ≥3 stepwise therapeutic interventions with grade b tumors was 9.5-fold higher as compared to grade a tumors ($P=0.0021$).

Discussion

While considered as benign in most cases (2), the efforts conducted for a better histopathological characterization of PT have considerably changed the diagnostic approach

Table 3 Evaluation of predictive factors for progression/recurrence of pituitary tumors in a uni- and multivariate analysis.

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* - value	HR	95% CI	*P*-value
Sex	1.44	0.96–2.12	0.0728	0.96	0.65–1.42	0.8448
Age (/10 years)	0.86	0.77–0.97	0.0147	0.83	0.72–0.95	0.0069
Size of the tumor						
Microadenoma (<10 mm)	1	–	–	1	–	–
Macroadenoma (≥10 mm)	2.70	1.42–5.09	0.0017	1.16	0.54–2.61	0.7037
MRI invasion (Knosp ≥ 3)						
No	1	–	–	1	–	–
Yes	7.40	4.77–11.38	<0.0001	2.82	1.39–6.62	0.0028
Histological subtype						
Immunonegative	1	–	–	1	–	–
LH/FSH	0.48	0.28–0.81	0.0093	0.64	0.39–1.03	0.0673
GH	0.54	0.31–0.96	0.0464	0.57	0.32–0.97	0.0399
GH/PRL	0.15	0.04–0.47	0.0004	0.22	0.06–0.58	0.0013
PRL	0.27	0.08–0.86	0.0356	0.35	0.09–1.00	0.0506
ACTH	0.66	0.38–1.14	0.1640	1.10	0.60–1.96	0.7575
Grade						
1a	1	–	–	1	–	–
1b	5.08	2.45–10.63	<0.0001	3.27	1.59–6.56	0.0017
2a	6.47	3.83–11.04	<0.0001	2.14	0.83–4.98	0.1101
2b	22.80	10.77–46.24	<0.0001	4.80	1.85–11.29	0.0021

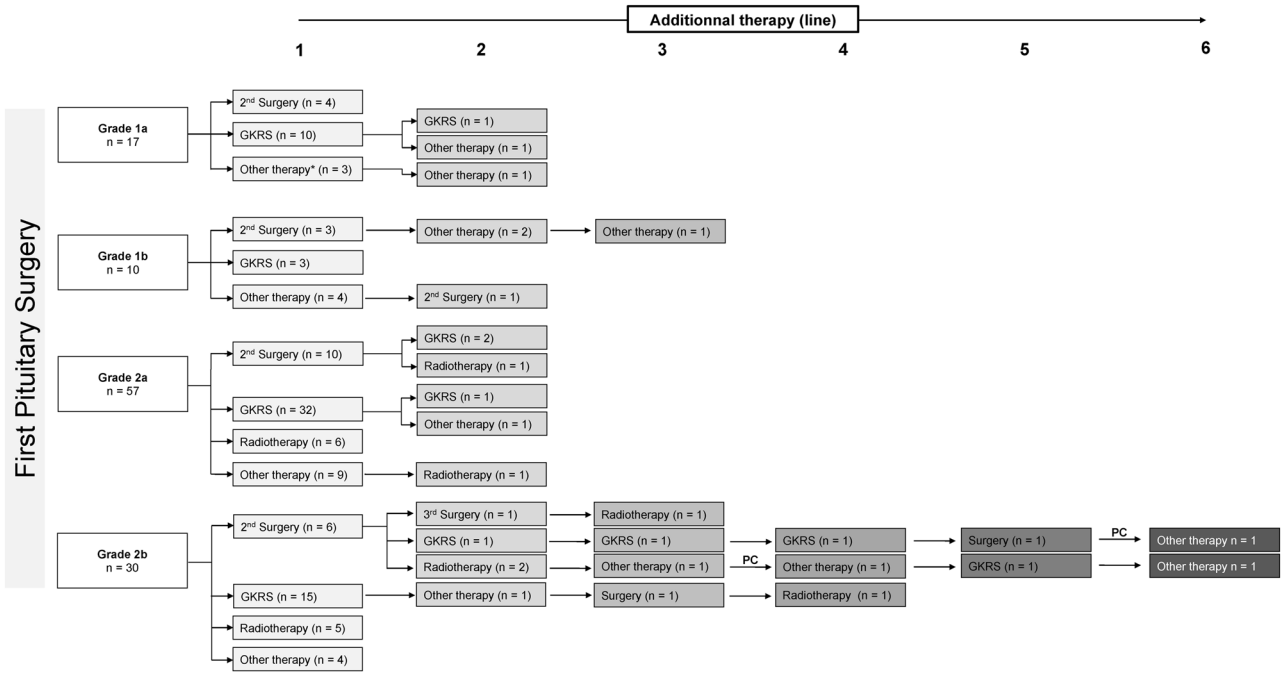


Figure 3

Therapeutic sequences following the first pituitary surgery. GKRS, gamma knife radiosurgery. Other therapy can include steroidogenesis inhibitors, somatostatin receptor ligands, and dopamine agonists alone or in combination. The abbreviation PC above the arrow means that the patient was diagnosed with pituitary carcinoma from this point.

of these lesions. By the analysis of proliferative markers, distinction between invasive and non-invasive tumors and, eventually, establishing a score based on a five-tiered classification, Trouillas and coworkers provided a reliable predictive grading system for the evolutionary risk of a PT (11). Three other independent cohort of patients validated this classification (14, 15, 16, 17), with a total of 2077 patients including our work. We here show that grade 2b tumors have an independent 4.8-fold increase risk of recurrence/progression with respect to grade 1a tumors. Moreover, progression-free survival was significantly shortened when the tumor was either invasive or proliferative as compared to their non-invasive and non-proliferative counterparts. Furthermore, the two patients who developed metastasis had grade 2b tumors, making this classification also a remarkable approach to the continuum that exists between aggressive tumors and carcinomas, a concept also known as the two sides of the same coin (18). With respective grade 1a, 1b, 2a and 2b of 50, 8.7, 33 and 8%, our cohort is roughly similar to the one published by Raverot and coworkers (15), strengthening its representativity as a population of patients from an expert pituitary center. However, our cohort also shows some differences. We had less LH/FSH immunopositive tumors because we classified a significant number of cases as immunonegative, a definition which should now apply to PT negative for mature hormones when the reactivity for transcription factors is unknown or not performed (19). In past studies, it is likely that immunonegative tumors were classified as null cell tumors, a subtype which exhibit features of tumors from the gonadotroph lineage (20). We also had fewer lactotroph tumors in our cohort, an obvious consequence of the surgical recruitment of our population and because medical therapy usually represents the first-line option for these tumors (21). However, in the study by Asioli and coworkers (14, 17), a quite high proportion of operated prolactinomas (up to 15%) was observed, which could be related to the fact that pituitary surgery is actively discussed in expert pituitary center as a first-line option for lactotroph tumors (22). We paid attention to distinguish silent and secreting GH or ACTH tumors and identified 19 (3.1%) of silent corticotroph tumors (siACTH), a prevalence close to the one observed (4.8%) in the literature (23). Recurrence rate of siACTH reached 47%, the highest in our study; however, only 3 out of 19 (15%) of these tumors were proliferative, and the remaining cases were grade 2a. These findings are in line with those of Lelotte and coworkers (16) and prompted us to speculate that relapse of siACTH

Table 4 Patients with proliferative tumors requiring more than three adjuvant therapies after surgery.

Patient	Sex	Age	Type of pituitary tumor				First surgery	Residue	Progression (time in months from first surgery)	Metastasis	Therapeutic sequences (time in months from first surgery)
			Size	Invasive	Subtype	Histopathology					
Pt. 1	F	50	Macro	Yes	ACTH	Ki67: 5% Mitotic count: 3/10 2b	STR	Yes	20 months	No	GKRS (20 months) Bilateral adrenalectomy (36 months) Medical therapy: DA and SRL Second surgery (45 months) Conformational radiotherapy (51 months) Temozolomide: 12 cycles (on going)
Pt. 2	M	44	Macro	No	ACTH	Ki67: 3% Mitotic count: 3/10 P53 positive 2b	GTR	No	14 months 72 months: liver and bone metastasis	Yes	Second surgery (14 months) Medical therapies: DA and SI Totosellar radiotherapy (18 months) Bilateral adrenalectomy (24 months) Radiofrequency ablation on liver metastasis and surgery/ radiotherapy on bone metastasis (72 months) Temozolomide Cyberknife (pituitary tumor residue) Bone radiotherapy (spine) Immunotherapy with ipilimumab and nivolumab (on going)
Pt. 3	F	31	Micro	No	ACTH	Ki67: 5% Mitotic count: 3/10 P53: positive 1b	GTR	No	25 months	No	Second surgery (25 months) Medical therapies: DA, SRL and SI Bilateral adrenalectomy (39 months) LFU: no residue
Pt. 4	F	51	Macro	Yes	ACTH + GH	Ki67: 7% Mitotic count: 3/10HPF 2b	STR	Yes	19 months 132 months: carcinoma meningitidis	Yes	Second surgery (19 months) GKRS (31 and 43 months) Third surgery (84 months) GKRS (96, 108 and 120 months) Temozolomide (132 months) Passed away (144 months)
Pt. 5	F	56	Macro	Yes	Negative	Ki67: 4% Mitotic count: 3/10HPF P53: positive 2b	STR	Yes	17 months	No	Second surgery (17 months) Third surgery (41 months) Conformational radiotherapy (53 months)

DA, dopamine agonist; GTR, gross tumor resection; SI, steroidogenesis inhibitor; SRL, somatostatin receptor ligand; STR, subtotal tumor resection.

is rather a consequence of their invasiveness more than a sustained proliferative activity an assumption supported by a recent work showing a highly activated epithelial-to-mesenchymal transition mechanism in siACTH (24). Likewise, the large study conducted by Langlois *et al.* over 39 cases of siACTH showed that all the tumor had Ki67 < 3% and only 1 had P53 positivity (23). At the clinical level, the young age has constantly been identified as an independent predictor of recurrence/progression in PT (15, 16), even though clear molecular explanations are lacking. Part of them could be that germline mutations in *AIP* and *MEN1* that are mainly found in young patients with macroadenomas (25) lead to a more aggressive phenotype, in particular for *AIP* (26) or because molecular mechanisms of pituitary tumorigenesis, when occurring at a young age, likely predispose to more aggressive tumors. This fact could explain why we observed a 17% decrease in the risk of recurrence for every decade achieved following the age at diagnosis of the patient.

Whether proliferation without invasion, or the other way around, is the most critical for tumor recurrence is still discussed. Our study shows that invasion was not significantly associated with a higher risk of recurrence/progression, a controversial finding in the literature (15, 16). However, we show that radiological invasion (Knosp ≥ 3) within the cavernous sinus invasion is associated with a high risk of recurrence, as was observed in a study that included more than 500 cases of PT (27). Conversely, our study also shows that grade 2a tumors that were considered invasive because of histological microinvasion had a significantly lower risk of recurrence/progression as compared to those with radiological invasion. This discrepant outcome, within the same grade of PT, should question the necessity to distinguish grade 2a tumors with radiological invasion from grade 2a tumors with microscopic invasion, especially when it comes to personalizing the follow-up of the patient. The proliferative status of the classification relies on the determination of Ki67, P53 and mitotic count which also raises questions. It is currently unknown whether positive P53 or high mitotic count really provide added values to a high Ki67 value. By using predictive models, Pappy II *et al.* showed that tumors exhibiting cavernous sinus invasion and high Ki67 had the worst outcome than tumor with high Ki67, mitotic count and positive P53 staining (27). A prospective study that will assess the evolutionary risk of PT stratified according to their Ki67, P53 and mitotic count respective values would undoubtedly mark a step forward to clarify this issue. Our study failed to identify the size as a predictive factor or progression/recurrence; however, this could be related to the mean maximal diameter or our cohort (only 20 ± 10.6 mm). Indeed, a previous study suggested that pituitary tumors that were invasive to the cavernous sinus with high Ki67 value were even further at risk of recurrence if their maximal diameter was ≥2.9 cm (27). This data could warrant clinicians to set up the patient's follow-up depending on the grade of the tumor and also its maximal diameter, especially if the latter exceeds 2.9 cm.

Applying a decision-making personalized medicine based on the tumor grade is questionable. To the best of our knowledge, our study is the first to put into perspective the therapeutic outcomes of the patient and the grade of the tumor. We show that progression/recurrence led to a new therapeutic intervention in 90% of patients and concerned 5.6% grade 1a, 18.9% grade 1b, 28.2% grade 2a and eventually, 73.2% grade 2b tumors, respectively. Interestingly, patients with proliferative tumors had a significantly higher risk to undergo adjuvant therapies following primary surgery even though, the treatment could have also been indicated because of persistent hypersecretion, a condition that was not necessarily related to tumor growth. Overall, the control of the disease was achieved in 85% of cases following this first-line additional therapy. Our study further demonstrates that GKRS and conventional radiotherapy succeed to control tumor progression in 91.6 and 100% of cases, respectively, including a majority of proliferative tumors. As such, five patients (less than 1% of the whole cohort) underwent three or more additional therapies and four of them had grade 2b tumors. Whether the existence of grade 2b PT residue at 3 months postoperative should lead to additional radiotherapy is a relevant question that would deserve to be seriously investigated. GKRS could be a suitable option (13), recent data suggesting its safety over neurocognitive consequences in the long term (28). Likewise, conventional radiotherapy, used for the treatment of pituitary tumors, slightly increased the risk of second brain tumors, however, with a much lower incidence than previously feared (29). Finally, due to its retrospective nature, our study has obvious inherent limitations. A non-negligible number of patients, especially those with acromegaly, were pretreated with SRL and/or dopamine agonist before surgery. To what degree this treatment impacts the proliferative activity of the pituitary tumor is questionable. Previous studies showed that SRL significantly decrease both the tumor volume and Ki-67 proliferation index in acromegalic patients (30, 31). This could result in an underestimation of grade b tumors and be the cause of the significantly lower risk of progression we observed in GH and GH/PRL subtypes.

However, our distribution of tumor grades was comparable to the one of other groups, who did not systematically use presurgical medical treatment. The retrospective data compendium did not allow us to get all the histopathological details of the tumors known to influence tumor growth or progression, like it was described for the granulation pattern of somatotropinomas. However, a recent study suggested that the granulation pattern of somatotropinomas was not an independent predictor of remission (32).

In conclusion, we validated that Trouillas' classification is a suitable grading system that should be evaluated in individual tumors for the identification of clinically aggressive tumors. Among the latter, grade 2b tumors expose the patient to repeated multimodal therapies that should raise the discussion of a precocious adjuvant therapy in a personalized medical approach.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EJE-220812.

Declaration of interest

The authors have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgement

The authors want to thank Pr. Nicolas Chevalier for his help in collecting the patient's data.

References

1 Lopes MBS. The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. *Acta Neuropathologica* 2017 **134** 521–535. (https://doi.org/10.1007/s00401-017-1769-8)

2 Melmed S. Pituitary-tumor endocrinopathies. *New England Journal of Medicine* 2020 **382** 937–950. (https://doi.org/10.1056/NEJMra1810772)

3 Asa SL, Casar-Borota O, Chanson P, Delgrange E, Earls P, Ezzat S, Grossman A, Ikeda H, Inoshita N, Karavitaki N *et al.* From pituitary adenoma to pituitary neuroendocrine tumor (PitNET): an International Pituitary Pathology Club proposal. *Endocrine-Related Cancer* 2017 **24** C5–C8. (https://doi.org/10.1530/ERC-17-0004)

4 Knosp E, Kitz K, Steiner E & Matula C. Pituitary adenomas with parasellar invasion. *Acta Neurochirurgica. Supplementum* 1991 **53** 65–71. (https://doi.org/10.1007/978-3-7091-9183-5_12)

5 Dhandapani S, Singh H, Negm HM, Cohen S, Anand VK & Schwartz TH. Cavernous sinus invasion in pituitary adenomas: systematic review and pooled data meta-analysis of radiologic criteria and comparison of endoscopic and microscopic surgery. *World Neurosurgery* 2016 **96** 36–46. (https://doi.org/10.1016/j.wneu.2016.08.088)

6 Dekkers OM, Karavitaki N & Pereira AM. The epidemiology of aggressive pituitary tumors (and its challenges). *Reviews in Endocrine and Metabolic Disorders* 2020 **21** 209–212. (https://doi.org/10.1007/s11154-020-09556-7)

7 Raverot G, Ilie MD, Lasolle H, Amodru V, Trouillas J, Castinetti F & Brue T. Aggressive pituitary tumours and pituitary carcinomas. *Nature Reviews. Endocrinology* 2021 **17** 671–684. (https://doi.org/10.1038/s41574-021-00550-w)

8 Al-Shraim M & Asa SL. The 2004 World Health Organization classification of pituitary tumors: what is new? *Acta Neuropathologica* 2006 **111** 1–7. (https://doi.org/10.1007/s00401-005-1093-6)

9 Miermeister CP, Petersenn S, Buchfelder M, Fahlbusch R, Lüdecke DK, Hölsken A, Bergmann M, Knappe HU, Hans VH, Flitsch J *et al.* Histological criteria for atypical pituitary adenomas - data from the German pituitary adenoma registry suggests modifications. *Acta Neuropathologica Communications* 2015 **3** 50. (https://doi.org/10.1186/s40478-015-0229-8)

10 Chiloiro S, Doglietto F, Trapasso B, Iacovazzo D, Giampietro A, Di Nardo F, de Waure C, Lauriola L, Mangiola A, Anile C *et al.* Typical and atypical pituitary adenomas: a single-center analysis of outcome and prognosis. *Neuroendocrinology* 2015 **101** 143–150. (https://doi.org/10.1159/000375448)

11 Trouillas J, Roy P, Sturm N, Dantony E, Cortet-Rudelli C, Viennet G, Bonneville JF, Assaker R, Auger C, Brue T *et al.* A new prognostic clinicopathological classification of pituitary adenomas: a multicentric case–control study of 410 patients with 8 years post-operative follow-up. *Acta Neuropathologica* 2013 **126** 123–135. (https://doi.org/10.1007/s00401-013-1084-y)

12 Bonneville JF. Magnetic resonance imaging of pituitary tumors. *Frontiers of Hormone Research* 2016 **45** 97–120. (https://doi.org/10.1159/000442327)

13 Castinetti F, Régis J, Dufour H & Brue T. Role of stereotactic radiosurgery in the management of pituitary adenomas. *Nature Reviews. Endocrinology* 2010 **6** 214–223. (https://doi.org/10.1038/nrendo.2010.4)

14 Asioli S, Righi A, Iommi M, Baldovini C, Ambrosi F, Guaraldi F, Zoli M, Mazzatenta D, Faustini-Fustini M, Rucci P *et al.* Validation of a clinicopathological score for the prediction of post-surgical evolution of pituitary adenoma: retrospective analysis on 566 patients from a tertiary care centre. *European Journal of Endocrinology* 2019 **180** 127–134. (https://doi.org/10.1530/EJE-18-0749)

15 Raverot G, Dantony E, Beauvy J, Vasiljevic A, Mikolasek S, Borson-Chazot F, Jouanneau E, Roy P & Trouillas J. Risk of recurrence in pituitary neuroendocrine tumors: a prospective study using a five-tiered classification. *Journal of Clinical Endocrinology and Metabolism* 2017 **102** 3368–3374. (https://doi.org/10.1210/jc.2017-00773)

16 Lelotte J, Mourin A, Fomekong E, Michotte A, Raftopoulos C & Maiter D. Both invasiveness and proliferation criteria predict recurrence of non-functioning pituitary macroadenomas after surgery: a retrospective analysis of a monocentric cohort of 120 patients. *European Journal of Endocrinology* 2018 **178** 237–246. (https://doi.org/10.1530/EJE-17-0965)

17 Guaraldi F, Zoli M, Righi A, Gibertoni D, Marino Picciola V, Faustini-Fustini M, Morandi L, Bacci A, Pasquini E, Mazzatenta D *et al.* A practical algorithm to predict postsurgical recurrence and progression of pituitary neuroendocrine tumours (PitNET)s. *Clinical Endocrinology* 2020 **93** 36–43. (https://doi.org/10.1111/cen.14197)

18 Trouillas J, Jaffrain-Rea ML, Vasiljevic A, Dekkers O, Popovic V, Wierinckx A, McCormack A, Petersenn S, Burman P, Raverot G *et al.* Are aggressive pituitary tumors and carcinomas two sides of the same coin? Pathologists reply to clinician's questions. *Reviews in Endocrine and Metabolic Disorders* 2020 **21** 243–251. (https://doi.org/10.1007/s11154-020-09562-9)

19 Asa SL, Mete O, Perry A & Osamura RY. Overview of the 2022 WHO classification of pituitary tumors. *Endocrine Pathology* 2022 **33** 6–26. (https://doi.org/10.1007/s12022-022-09703-7)

20 Neou M, Villa C, Armignacco R, Jouinot A, Raffin-Sanson ML, Septier A, Letourneur F, Diry S, Diedisheim M, Izac B *et al.* Pangenomic classification of pituitary neuroendocrine tumors. *Cancer Cell* 2020 **37** 123–134.e5. (https://doi.org/10.1016/j.ccell.2019.11.002)

21 Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, Wass JA & Endocrine Society. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 273–288. (https://doi.org/10.1210/jc.2010-1692)

22 Baussart B, Villa C, Jouinot A, Raffin-Sanson ML, Foubert L, Cazabat L, Bernier M, Bonnet F, Dohan A, Bertherat J *et al.* Pituitary surgery as alternative to dopamine agonists treatment for microprolactinomas: a cohort study. *European Journal of Endocrinology* 2021 **185** 783–791. (https://doi.org/10.1530/EJE-21-0293)

23 Langlois F, Lim DST, Yedinak CG, Cetas I, McCartney S, Cetas J, Dogan A & Fleseriu M. Predictors of silent corticotroph adenoma recurrence; a large retrospective single center study and systematic literature review. *Pituitary* 2018 **21** 32–40. (https://doi.org/10.1007/s11102-017-0844-4)

24 Zhang D, Hugo W, Bergsneider M, Wang MB, Kim W, Vinters HV & Heaney AP. Single-cell RNA sequencing in silent corticotroph tumors confirms impaired POMC processing and provides new insights into their invasive behavior. *European Journal of Endocrinology* 2022 **187** 49–64. (https://doi.org/10.1530/EJE-21-1183)

25 Cuny T, Pertuit M, Sahnoun-Fathallah M, Daly A, Occhi G, Odou MF, Tabarin A, Nunes ML, Delemer B, Rohmer V *et al.* Genetic analysis in young patients with sporadic pituitary macroadenomas: besides AIP don't forget MEN1 genetic analysis. *European Journal of Endocrinology* 2013 **168** 533–541. (https://doi.org/10.1530/EJE-12-0763)

26 Daly AF, Tichomirowa MA, Petrossians P, Heliövaara E, Jaffrain-Rea ML, Barlier A, Naves LA, Ebeling T, Karhu A, Raappana A *et al.* Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** E373–E383. (https://doi.org/10.1210/jc.2009-2556)

27 Pappy AL, Savinkina A, Bicknese C, Neill S, Oyesiku NM & Ioachimescu AG. Predictive modeling for pituitary adenomas: single center experience in 501 consecutive patients. *Pituitary* 2019 **22** 520–531. (https://doi.org/10.1007/s11102-019-00982-8)

28 Castinetti F, Caron P, Raingeard I, Amodru V, Albarel F, Morange I, Chanson P, Calvo J, Graillon T, Baumstarck K *et al.* Lack of delayed neurocognitive side effects of gamma Knife radiosurgery in acromegaly: the Later-Ac study. *European Journal of Endocrinology* 2021 **186** 37–44. (https://doi.org/10.1530/EJE-21-0826)

29 Hamblin R, Vardon A, Akpalu J, Tampourlou M, Spiliotis I, Sbardella E, Lynch J, Shankaran V, Mavilakandy A, Gagliardi I *et al.* Risk of second brain tumour after radiotherapy for pituitary adenoma or craniopharyngioma: a retrospective, multicentre, cohort study of 3679 patients with long-term imaging surveillance. *Lancet. Diabetes and Endocrinology* 2022 **10** 581–588. (https://doi.org/10.1016/S2213-8587(22)00160-7)

30 Caron PJ, Bevan JS, Petersenn S, Flanagan D, Tabarin A, Prévost G, Maisonobe P, Clermont A & PRIMARYS Investigators. Tumor shrinkage with lanreotide Autogel 120 mg as primary therapy in acromegaly: results of a prospective multicenter clinical trial. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 1282–1290. (https://doi.org/10.1210/jc.2013-3318)

31 Selek A, Cetinarslan B, Canturk Z, Tarkun I, Hanazay Y, Vural C, Anik I & Ceylan S. The effect of somatostatin analogues on Ki-67 levels in GH-secreting adenomas. *Growth Hormone and IGF Research* 2019 **45** 1–5. (https://doi.org/10.1016/j.ghir.2019.01.001)

32 Swanson AA, Erickson D, Donegan DM, Jenkins SM, Van Gompel JJ, Atkinson JLD, Erickson BJ & Giannini C. Clinical, biological, radiological, and pathological comparison of sparsely and densely granulated somatotroph adenomas: a single center experience from a cohort of 131 patients with acromegaly. *Pituitary* 2021 **24** 192–206. (https://doi.org/10.1007/s11102-020-01096-2)