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

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Abstract

Objective and design: A clinicopathological score has been proposed by Trouillas *et al.* to predict the evolution of pituitary adenomas. Aim of our study was to perform an independent external validation of this score and identify other potential predictor of post-surgical outcome.

Methods: The study sample included 566 patients with pituitary adenomas, specifically 253 FSH/LH-secreting, 147 GH-secreting, 85 PRL-secreting, 72 ACTH-secreting and 9 TSH-secreting tumours with at least 3-year post-surgical follow-up.

Results: In 437 cases, pituitary adenomas were non-invasive, with low (grade 1a: 378 cases) or high (grade 1b: 59 cases) proliferative activity. In 129 cases, tumours were invasive, with low (grade 2a: 87 cases) or high (grade 2b: 42 cases) proliferative activity. During the follow-up (mean: 5.8 years), 60 patients developed disease recurrence or progression, with a total of 130 patients with pituitary disease at last follow-up. Univariate analysis demonstrated a significantly higher risk of disease persistence and recurrence/progression in patients with PRL-, ACTH- and FSH/LH-secreting tumours as compared to those with somatotroph tumours, and in those with high proliferative activity (grade 1b and 2b) or >1 cm diameter. Multivariate analysis confirmed tumour type and grade to be independent predictors of disease-free survival. Tumour invasion, Ki-67 and tumour type were the only independent prognostic factors of disease-free survival.

Conclusions: Our data confirmed the validity of Trouillas' score, being tumour type and grade independent predictors of disease evolution. Therefore, we recommend to always consider both features, together with tumour histological subtype, in the clinical setting to early identify patients at higher risk of recurrence.

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Introduction

Pituitary adenomas (PAs) are relatively common intracranial tumours arising from the adenohypophysis and accounting for 10–15% of all intracranial neoplasms (1, 2, 3, 4). Although typically benign under a histological point of view, they can

exhibit an aggressive clinical and radiological behaviour, characterised by rapid growth with gross invasion of the surrounding tissues, together with resistance, or early recurrence, after treatment (4, 5, 6, 7, 8). Therefore, it has

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been recently proposed to rename these lesions as pituitary neuroendocrine tumours (PitNET) (3). Only rarely they metastasise through the cerebrospinal fluid or systemically and are then considered 'pituitary carcinomas' (6, 7).

The term 'atypical adenoma' defined in the 2004 WHO classification (8) as an 'adenoma with high mitotic index, Ki-67 labelling index (LI) $\geq 3\%$ and strong nuclear p53 protein immunoexpression' was abandoned in the 2017 WHO classification, since a definite correlation between histological diagnosis and clinical behaviour could not be established (1). However, the 2017 WHO classification, still suggests to divide PAs between 'low' and 'high risk' based on their proliferative activity (1). Recently, Trouillas *et al.* (5) proposed a clinicopathological score based on the evidence of tumour invasion and proliferation and identifying the following features as risk criteria: mitoses $\geq 2/10$ HPF, Ki-67 $\geq 3\%$ and p53 expression >10 strongly positive nuclei/10 HPF. Since in most of the cases invasion can only be evaluated by MRI and/or surgical findings, the proposed score requires a multidisciplinary approach involving the pathologist, the radiologist, the endocrinologist and the neurosurgeon. In brief, the authors proposed a '5 grades score': grade 1a, non-invasive and low proliferative tumour; grade 1b, non-invasive and high proliferative tumour; grade 2a, invasive and low proliferative tumour; grade 2b, invasive and high proliferative tumour and grade 3, metastatic tumour (cerebrospinal or systemic metastases). The score was validated by the same group on a retrospective multicentric case-control study, which included 410 patients with 8-year post-operative follow-up (5). Tumour invasion with high proliferation were predictors of aggressive clinical behaviour, in terms of disease-free survival and recurrence/progression-free status (5). These preliminary findings were confirmed by a subsequent prospective independent cohort of 240 patients with a mean follow-up of 3.5 years (2).

The aim of our study was to analyse several histopathological, clinical and radiological criteria in our large cohort of patients operated for PAs to validate the classification proposed by Trouillas *et al.* (5) and to identify other potential factors predicting post-surgical disease evolution.

Subjects and methods

Study sample

The study included all consecutive patients operated of PAs at the Pituitary Unit, IRCCS Istitutedelle Scienze Neurologiche, Bologna, Italy, between January 1998 and

December 2012. Inclusion criteria were (1) availability of tissue slides and paraffin blocks of sufficient size to allow morphological and immunohistochemical characterisation; (2) no history of radiation therapy before or after surgery in the absence of a demonstrated tumour progression; (3) available pre- and post-surgery clinical information, including complete basal and stimulated (when appropriate) hormonal evaluation and neuro-imaging data and (4) minimum post-surgical follow-up of 3 years. Existing slides were retrieved from the pathology archives and reviewed.

All PAs were removed by endoscopic endonasal surgery by two expert neurosurgeons (M Z, D M). One-hundred and eight cases had been previously reported in other studies. For these cases, we retrieved clinical information from the last follow-up, dating at the time of publication, with the same methods applied to the investigation of new cases (see 'Materials and methods' section). For all cases, histological and radiological revision was performed applying the criteria suggested by Trouillas' *et al.* (9, 10, 11, 12).

According to pre-operative hormonal and clinical features, tumours were classified into clinically 'functioning' and 'non-functioning'. Based on their size in the pre-operative MRI, tumours were classified into 'micro-' (<10 mm) and 'macroadenomas' (≥ 10 mm). Tumour invasion was suspected on pre-operative MRI for cases with grade of Knosp >3 , although only cases with demonstrated invasion of the cavernous sinus and/or sphenoidal bone during surgical exploration and/or pathological evaluation were considered 'invasive' (13).

Follow-up monitoring included post-operative MRI, endocrinological and neurological evaluations, performed at 3 months after surgery and then at variable intervals, depending on the clinical, biochemical and neuroradiological findings. The degree of tumour removal was evaluated on post-operative MRI and defined as 'radical' in the absence of visible remnant; 'subtotal' for remnant $<20\%$ of the original mass or 'partial' if $>20\%$ of the original mass. Normalisation of hormone hypersecretion (or disease control) was defined according to post-operative basal hormone secretion, and stimulated/inhibited function, whenever necessary. In case of persistent hormone hypersecretion, targeted medical therapy was prescribed. 'Surgical cure' was defined by radical resection in non-functioning tumours, together with the normalisation of hormone secretion in functioning ones. Conversely, non-functioning tumours with a stable remnant at follow-up or functioning ones with control of hormonal secretion by medical therapy

were considered 'controlled'. 'Relapse' was defined as the recurrence or increase of remnant dimensions in non-functioning PAs or the recurrence of hormone hypersecretion (treatment escape in case of medical treatment) in functioning tumours (5, 9).

The present study was approved by an inter-hospital Ethical Committee of Bologna city (protocol number CE17143, dating February 20th, 2018). Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

Immunohistochemistry

All slides were reviewed and classified by three expert pituitary pathologists (A S, A R and M P F) according to the recently published WHO classification of endocrine organs tumours (1). Tumours expressing multiple hormones were classified according to the mostly represented hormone immunoreactivity (5). Ki-67 LI was expressed as the percentage of tumour cells with positive nuclei. Following the criteria proposed by Trouillas *et al.* (5), p53 protein stain was considered positive for >10 strongly positive nuclei per 10 high power fields (HPF 0.30 mm², 400× magnification) (5). Similar to other endocrine neoplasms, Ki-67 LI and p53 detection was based on the evaluation of cells in the areas with the highest nuclear labelling ('hotspots'). A digital camera (Olympus Q-colour 3, Tokyo, Japan) with area-based image analysis software (Dot-Slide 1.2 version) was used to minimise inter- and intra-observer variability. Immunostained sections were screened under optical microscope and the areas containing the greatest number of Ki-67-stained and p53-stained nuclei were outlined with a slide marker, allowing easy localisation during image analysis, as previously described (9). PAs subtyping by immunohistochemistry was performed according to the criteria proposed by Trouillas *et al.* and recently incorporated in the WHO classification (1, 5). Silent ACTH tumours ($n=14$) and one ACTH tumour secondary to Nelson syndrome were grouped together with ACTH adenomas.

Statistical analysis

Categorical variables were summarised using absolute and relative frequencies, while quantitative variables were summarised using mean and standard deviation.

Disease-free survival distribution was estimated using Kaplan–Meier product limit.

Gender, age (treated as a continuous variable), tumour size, tumour type, mitotic index, invasion, Ki-67 LI, p53

expression and tumour grade were analysed as possible predictors of outcomes. The log-rank test was used to compare survival curves between groups. Cox proportional hazards models were used to estimate the hazard ratio and confidence intervals for each risk factor. The proportional hazards assumption was tested using Schoenfeld residuals. Multivariable Cox proportional hazards regression models were used to adjust for explanatory variables.

For all tests, significance was defined as $P<0.05$. Statistical analysis was performed using IBM SPSS version 23.0 and Stata 12.

Results

Demographic, clinical and pathologic features

Main sample demographic, clinical and pathologic features at baseline and follow-up period are summarised in Table 1. The study sample included 566 patients (310 F, 54.8%) with PAs treated by trans-sphenoidal endoscopic endonasal surgery. Age at the time of first surgery was 49.8 ± 15.5 years; median 50; range 12–85). At the pre-operative MRI, most tumours ($n=440$; 77.7%) were macroadenomas. According to the previously mentioned criteria, 129 (22.8%) tumours were considered invasive, with surgical and/or pathological demonstrated invasion of the cavernous ($n=109$, 19%) or sphenoid ($n=20$; 3.5%) sinus.

Based on the immunohistochemical data, the study sample included 253 FSH/LH-, 147 GH-, 85 PRL-, 72 ACTH- (14 silent) and 9 TSH-secreting tumours. The group of GH-secreting tumours was made of 45 sparsely granulated and 83 densely granulated lesions; 17 were mixed GH- and PRL-secreting tumours, while two presented plurihormonal secretion. Two hundred and fifty-three FSH/LH PAs consisted of 183 null cell tumours (of these, 19 were of oncocytic variant) and 70 gonadotroph cell lesions.

A high mitotic index (≥ 2 each 10 HPF) was found in 107 cases (18.9%). Mean Ki-67 LI was 2.1% (range 0.1–21); 105 (18.6%) cases showed a Ki-67 LI value $\geq 3\%$. P53 protein expression was positive in 133 (23.5%) cases. According to the Trouillas' classification criteria, 378 (66.8%) tumours were grade 1a; 59 (10.4%) grade 1b; 87 (15.4%) grade 2a and 42 (7.4%) grade 2b. Tumour grade distribution for each tumour subtype defined by immunohistochemistry is presented in Table 2.

Mean follow-up was 5.8 years (range 3–17.7 years). During the follow-up period, 60 (10.6%) patients developed tumour recurrence after apparent gross total resection or disease progression after subtotal surgical resection.

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Table 1 Main sample demographic, clinical and pathologic features at baseline and follow-up.

	Total, n (%)	Disease-free, n (%)	
		Yes	No
Gender			
Male	256 (45.2)	194 (44.5)	62 (47.7)
Female	310 (54.8)	242 (55.5)	68 (52.3)
Age (years) mean $\pm$ s.d.	49.8 $\pm$ 15.5	49.9 $\pm$ 15.3	49.2 $\pm$ 16.0
Tumour size			
Micro	126 (22.3)	108 (24.8)	18 (13.8)
Macro	440 (77.7)	328 (75.2)	112 (86.2)
Invasion			
No	437 (77.2)	354 (81.2)	83 (63.8)
Yes	129 (22.8)	82 (18.8)	47 (36.2)
Tumor type			
GH	147 (26)	121 (27.8)	26 (20)
PRL	85 (15)	59 (13.5)	26 (20)
ACTH	72 (12.7)	55 (12.6)	17 (13.1)
TSH	9 (1.6)	8 (1.8)	1 (0.7)
FSH/LH	253 (44.7)	193 (44.3)	60 (46.2)
Mitoses			
<2	459 (81.1)	363 (83.3)	96 (73.8)
≥ 2	107 (18.9)	73 (16.7)	34 (26.2)
Ki-67			
<3%	461 (81.4)	373 (85.6)	88 (67.7)
$\geq 3\%$	105 (18.6)	63 (14.4)	42 (32.3)
p53			
Negative	433 (76.5)	342 (78.4)	91 (70)
Positive	133 (23.5)	94 (21.6)	39 (30)
Grade			
1a: Non-invasive	378 (66.8)	313 (71.8)	65 (50)
1b: Non-invasive, proliferative	59 (10.4)	41 (9.4)	18 (13.8)
2a: Invasive	87 (15.4)	66 (15.1)	21 (16.2)
2b: Invasive, proliferative	42 (7.4)	16 (3.7)	26 (20)

The mean time of recurrence/progression was 5.4 years (range 4 months to 17.5 years). At the last follow-up, 130 (22.9%) patients showed disease persistence, while the remaining were disease free.

Disease-free survival (DFS)

The disease-free survival distribution is summarised in Table 3 ref. cat., reference category. Four, six, eight and ten-year DFS was 86.8, 80.9, 75.1 and 63.4%, respectively. The disease-free survival, stratified by tumour subtype is presented in Table 4.

Univariate Cox regression models on the determinants of disease outcome showed that patients

with macroadenoma had a 81% higher risk of persistent disease after surgery compared with patients with microadenoma (HR=1.810, $P=0.02$) and that patients with invasive tumours had a higher risk of recurrence/progression than patients with non-invasive tumours (HR=2.258, $P<0.001$). A significantly higher risk of persistent disease after surgery was found in patients with PRL- (HR=2.968, $P<0.001$), ACTH- (HR=2.336, $P=0.009$) and FSH/LH-secreting (HR=2.276, $P=0.001$) tumour type compared with patients with somatotroph tumour. Tumours with a number of mitoses $\geq 2/10$ HPF had a higher the risk of recurrence/progression as compared with those with a lower number of mitoses (HR=2.215, $P<0.001$). Patients with Ki-67 LI $\geq 3\%$ and with high

Table 2 Tumour grade distribution for each tumour subtype defined by immunohistochemistry.

Grade	Tumour subtype				
	GH (n = 147)	PRL (n = 85)	ACTH (n = 72)	FSH/LH (n = 253)	TSH (n = 9)
1a	102 (69.4%)	45 (52.9%)	47 (65.3%)	179 (70.7%)	5 (55.6%)
1b	12 (8.2%)	12 (14.1%)	15 (20.8%)	19 (7.5%)	1 (11.1%)
2a	25 (17%)	15 (17.6%)	4 (5.6%)	40 (15.9%)	3 (33.3%)
2b	8 (5.4%)	13 (15.4%)	6 (8.3%)	15 (5.9%)	0

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Table 3 Predictors of disease-free status over the entire follow-up as defined by univariate Cox regression models.

	HR (95% CI)	P value
Gender		
Male (ref. cat.)	1	
Female	0.903 (0.640;1.275)	0.564
Age (years) mean $\pm$ s.d.	1.000 (0.989;1.011)	0.992
Tumour size		
Micro (ref. cat.)	1	
Macro	1.810 (1.099;2.979)	0.020
Invasion		
No (ref. cat.)	1	
Yes	2.258 (1.575;3.236)	<0.001
Tumour type		
GH (ref. cat.)	1	
PRL	2.968 (1.672;5.270)	<0.001
ACTH	2.336 (1.233;4.426)	0.009
TSH	1.125 (0.151;8.393)	0.909
FSH/LH	2.276 (1.389;3.731)	0.001
Mitoses		
<2	1	
≥ 2	2.215 (1.481;3.312)	<0.001
Ki-67		
<3%	1	
$\geq 3\%$	2.293 (2.005;4.260)	<0.001
p53		
Negative	1	
Positive	1.696 (1.162;2.478)	0.006
Grade		
1a (ref. cat.)	1	
1b	2.597 (1.531;4.408)	<0.001
2a	1.579 (0.963;2.592)	0.070
2b	5.516 (3.471;8.766)	<0.001

expression of p53 protein had a higher risk of recurrence/progression as compared with patients with Ki-67 LI <3% (HR=2.293, $P<0.001$) and with low p53 expression (HR=1.696, $P=0.006$), respectively. The hazard ratios for grades 1b and 2b were significantly higher compared with grade 1a (HR=2.597, $P<0.001$; HR=5.516, $P<0.001$). No statistically effect of gender or age on DFS was found (Table 3).

According to multivariate Cox regression analysis, which included tumour size, type and grade (the last incorporating invasiveness and proliferation) (Table 5A), tumour type and grade remained independent predictors of DFS, while tumour size was not. In particular, tumour grade 1b and 2b were independent predictors of worse outcome (HR=2.441, $P=0.001$ for grade 1b; HR=5.005, $P<0.001$ for grade 2b).

Considering every single variable, multivariate Cox regression analysis showed tumour invasion (HR=1.926; $P=0.001$), Ki-67 $\geq 3\%$ (HR=2.290; $P=0.003$) and type to be independent prognostic factor of DFS (Table 5B). Specifically, the risk of recurrence/progression was

significantly higher in PRL- (HR=2.428; $P=0.003$), ACTH- (HR=2.242; $P=0.018$) and FSH/LH-secreting tumours (HR=2.377; $P=0.002$) as compared with GH tumour. The grade distribution for sparsely and densely granulated GH-secreting tumours is presented in Table 6. Finally, sparsely granulated PAs were associated with a worse prognosis as compared to densely granulated ($P=0.005$ according to Pearson chi-square test).

Discussion

Our study demonstrated the role of histological type, grade of invasion and proliferation in the determination of the post-surgical outcome of PAs based on the retrospective analysis of more than 500 cases primary treated with surgery.

PAs are the most common pituitary disorders and are, by definition, histologically benign. However, in a variable percentage of cases, they present an aggressive behaviour with the invasion of surrounding structures, together with partial/no response to treatment or recurrence after surgery, and, more rarely, distant metastases (pituitary carcinomas) (1). Therefore, the identification of prognostic factors of outcome in this field would be of primary importance to provide patients the most appropriate follow-up based on predefined risk of recurrence/resistance to treatment. Unfortunately, to date, studies focusing on this issue are scanty.

In 2004, the WHO classification of Tumours of Endocrine organs proposed the distinction between 'typical' and 'atypical' adenomas, that has been removed

Table 4 Disease-free survival according to tumour subtype.

	HR (95% CI)	P
GH (1a reference category)		
1b	2.093 (0.597;7.340)	0.248
2a	1.472 (0.558;3.887)	0.435
2b	4.828 (1.345;17.325)	0.016
PRL (1a reference category)		
1b	0.963 (0.262;3.539)	0.955
2a	1.038 (0.286;3.764)	0.955
2b	4.573 (1.871;11.177)	0.001
ACTH (1a reference category)		
1b	10.044 (2.691;37.491)	0.001
2a	9.155 (1.657;50.587)	0.011
2b	3.310 (0.712;15.394)	0.127
FSH/LH (1a reference category)		
1b	3.029 (1.165;7.873)	0.023
2a	1.949 (0.947;4.011)	0.070
2b	6.501 (3.098;13.644)	<0.001
TSH, model did not converge.		

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Table 5 Predictors of disease-free status over the entire follow-up as defined by multivariate Cox regression analysis including tumour size, type, grade (incorporating invasiveness and proliferation), tumour size, type, number of mitoses, Ki-67 index and p53 expression.

	HR (95% CI)	P
A: Independent predictors of DFS		
Tumour size		
Micro	1	
Macro	1.251 (0.687;2.281)	0.464
Tumour type		
GH	1	
PRL	2.386 (1.328;4.285)	0.004
ACTH	2.101 (1.076;4.102)	0.03
TSH	0.861 (0.115;6.468)	0.884
FSH/LH	2.302 (1.344;3.943)	0.002
Grade		
1a	1	
1b	2.441 (1.419;4.198)	0.001
2a	1.661 (0.995;2.773)	0.052
2b	5.005 (3.030;8.265)	<0.001
B: Independent prognostic factors of DFS		
Size		
Micro	1	
Macro	1.300 (0.710;2.382)	0.395
Invasion		
No	1	
Yes	1.926 (1.301;2.850)	0.001
Tumor type		
GH	1	
PRL	2.428 (1.348;4.372)	0.003
ACTH	2.242 (1.146;4.387)	0.018
FSH/LH	2.377 (1.386;4.078)	0.002
Mitoses		
<2	1	
≥2	1.101 (0.654;1.852)	0.717
Ki-67		
<3%	1	
≥3%	2.290 (1.333;3.932)	0.003
p53		
Negative	1	
Positive	0.933 (0.586;1.485)	0.769

in the 2017 WHO classification for the absence of clinicopathological correlations (4, 8, 9, 14, 15, 16, 17, 18, 19, 20), leaving place to the cell lineage origin as a main criteria of classification (1).

Moreover, in the 2004 WHO classification, high mitotic index and a Ki-67 LI ≥3% were considered effective prognostic markers of PAs (4, 5, 8, 9, 14, 15, 16, 17, 18, 19, 20, 21). Expression of p53 was also included among potential outcome predictors (8), but is remained unclear whether its expression was an independent factor of aggressive behaviour because of the different cut-offs used in literature to differentiate tumours with low and high risk of recurrence/progression together with

the high inter-observer variability (1, 4, 5, 9, 18). Rapid growth, radiological evidence of invasion and high Ki-67 proliferation index were later suggested as features of clinically aggressive tumours, implying a closer follow-up in these cases. Recently, precise cut-off for the number of mitoses and p53 expression have been determined to define tumours at high risk of recurrence (4, 5, 8, 9, 14, 15, 16, 17, 18, 19, 20, 21).

In 2013, Trouillas *et al.* (5) proposed a clinicopathological classification, based on the evaluation of tumour invasion – defined at MRI and during surgery – and proliferation, through which it was possible to identify ‘5 grades’ of PAs. This classification was validated by the same group by a retrospective study that included 213 patients treated with surgery with a mean post-operative follow-up of 3.5 years (2). The validity of Trouillas’ classification to define PAs prognosis has been recently underlined by Lelotte *et al.* in the setting of gonadotroph tumours (22).

Our retrospective analysis, performed on 566 patients with all histotypes of PAs, confirmed the prognostic value of Trouillas’ classification, thus underlying the importance of a grading system that includes indexes of proliferation and invasiveness to predict post-surgical risk of disease persistence and recurrence/progression. In our paper, we assessed the invasiveness of these tumours not basing on the neuroradiological suspect of pre-operative invasion evaluated at MRI, but on the real inspection. Indeed, as stated by Micko *et al.* we believe that the real advantage of endoscopic endonasal technique is the great possibility to inspect the surgical cavity, achieving a direct evaluation of the real tumour invasion of cavernous sinus or other structures (23). Conversely, it has been demonstrated the any neuroradiological classification to determine the invasiveness of pituitary adenomas, such as Knosp grade, may not perfectly correlate with its real infiltration of cavernous sinus (23, 24). Moreover, in line with Trouillas *et al.* (5, 14), we also demonstrated the primary importance of tumour histotype in the prediction of treatment outcome, since it appears the best clinical predictor of disease-free survival, based on both uni- and multivariate Cox

Table 6 Grade distribution for GH-secreting tumours classified into sparsely and densely.

Grade	Sparsely granulated	Densely granulated
1a	30	60
1b	4	7
2a	8	13
2b	3	3

regression analyses. Specifically, patients with GH-secreting tumours had a significantly lower risk of disease persistence/recurrence with respect to all other types of PAs.

In our series (for unknown reasons), the ratio between densely ($n=86$) and sparsely ($n=45$) granulated somatotroph PAs was 1.86, which is higher than reported in other studies (4, 15, 25). On the other hand, we confirmed literature data about the more aggressive behaviour of the sparsely granulated variant (1, 6), associated with a significantly lower rate of disease-free survival with respect to densely granulated tumours ($P=0.005$ according to Pearson chi-square test).

A limitation of our study is the relatively short follow-up time (median 5.8 years), since pituitary tumours can take many years to recur or regrow (2). This difference is evident in our lower frequency of recurrence or progression events (10.6%) and of patients with evidence of disease (22.9%) compared with the study by Trouillas *et al.* (mean follow-up 11.1 years, recurrence 30.5%, persistent disease 52.4%). Despite this wide difference in terms of follow-up, the Ki-67, p53 and mitotic rate cut-off values proposed by Trouillas *et al.* (5) are confirmed in our study, and consequently a more precise categorisation of PAs in terms of their values may be possible. In addition, these cut-off values should also simplify and standardise the pathological analysis of adenomas, making comparisons across study cohorts possible. As reported by Raverot *et al.* (2), this classification should be further refined by other studies considering rarer tumours that those included herein, in particular specific morphological subtypes that have demonstrated to be prone to aggressive behaviour (such as Crooke cell adenomas, silent corticotroph adenoma, plurihormonal PIT1-positive adenoma).

In conclusion, we observed that Trouillas' classification essentially based on tumour proliferation (i.e. mitotic count and Ki-67 LI) and adenoma invasion should be evaluated in individual tumours for identification of clinically aggressive tumours. Tumour grade however is not the only independent parameter to discriminate cases with a high risk of recurrence/progression and of post-operative complete remission and should be integrated with the hormonal subtype.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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