Original Article

New Diagnostic Strategy for Atypical Pituitary Adenomas: Clinical and Histopathological Score

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ABSTRACT

Background: Currently, prognosis remains the major challenge of the adenomatous pituitary pathology. According to the World Health Organization (WHO), pituitary tumours are classified into typical adenoma, atypical adenoma and carcinoma. Given that the prediction of the behaviour of these tumours remains a major clinical and anatomo-pathological challenge, we propose a new diagnostic strategy to orient prognosis and therapy of these tumours, based on a multiparameter system, as well as a simple clinico-laboratory and radio-histopathologic diagnostic algorithm.

Methods: To validate the method, we have applied it retrospectively to a series of 243 pituitary adenomas (diagnosed according to the 2004 WHO classification on tumours of endocrine organs), operated by transsphenoidal via between 2004 and 2014, at Centro Hospitalar Lisboa Norte, the largest reference centre in Portugal.

Result: A hundred twentynine had a follow-up of at least 5 years in order to evaluate recurrences. While 6.2% of typical adenomas recurred, among the atypical the recurrence rate was 68.8%.

Conclusion: With this work we intend to provide a more specific differentiating system of possible malignancy, to early identify probable cases of poor evolution, which could be very useful in clinical practice.

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Introduction

Tumours of the pituitary gland and sellar region represent approximately 10 to 15% of all brain tumours.[1] In fact, pituitary adenomas (PA) represent the third most common primary intracranial tumour in neurosurgery, outnumbered by gliomas and meningiomas.[1] As a result of the extensive use of neuroimaging studies, asymptomatic and incidental PA ("incidentalomas") are increasingly common.[2,3] In a recent review of autopsy and magnetic resonance imaging (MRI) studies, the estimated overall prevalence of PA was 16.7%.[4] Recent studies show an increase in the PA prevalence up to four times above that previously thought.[5,6]

Although considered as benign, some PA are locally invasive and cause significant morbidity and mortality.[7,8] Other epithelial tumours classified as malignant neoplasm, for instance, skin basal cell carcinoma, although widely invasive rarely metastasize. In contrast, some aggressive pituitary tumours cause significant morbidity related to hormonal hypo or hypersecretion, may invade brain structures, cause blindness and cranial nerve paralysis; some may require radiation therapy and, ultimately, may be lethal, despite being considered histologically benign.[9] More than a decade after the last classification of the World Health Organization (WHO), a reassessment of the definition, classification and malignancy criteria of pituitary neoplasms seems appropriated, specifically for PA considered "atypical".

Since the first morphological classification proposed by Cushing in 1912, many attempts to histologically classify PA have been made. Initial classifications were based on the cellular tinctorial properties distinguishing acidophilic, basophilic and chromophobic adenomas; however, this staining classification does not correlate clinically with the functional characteristics of these tumours. Currently, classification of PA is based on histological criteria, mainly immunohistochemical (the gold standard of diagnosis) and ultrastructural, also taking into account clinical presentation, biochemical information, imaging techniques and surgical findings. Electron microscopy, an expensive and time-consuming technique, is rarely performed today.[10]

The current WHO classification of endocrine tumours of the pituitary gland, classifies them as typical adenoma (ICD 8272/0), atypical adenoma (ICD 8272/1) and pituitary carcinoma (ICD 8272/3).[11] However, differences between "typical" and "atypical" adenoma are not clearly established, and there are no morphological criteria to distinguish locally aggressive atypical adenomas from carcinomas, when the tumour is limited to the sella turcica.[12] Most of PA are typical, with "bland" histological features, rare mitotic figures and a proliferative index (Ki67) lower than 3%. The mechanism of PA progression to more aggressive and invasive tumours is not fully elucidated; in fact a *continuum* from "typical" to "atypical" adenoma and carcinoma has not been demonstrated, as is well stablished for other types of epithelial tumours, like the adenoma-carcinoma intestinal sequence. Atypical PA exhibit a borderline or uncertain behaviour, with atypical morphological characteristics suggestive of aggressive behaviour (such as locally invasive growth), a high mitotic index, a cell proliferation index (Ki67) above 3% and extensive immunostaining for p53 protein.[11] They are not as uncommon as previously thought.[13,14]

Pituitary carcinomas are rare, representing 0.2% of pituitary tumours, in part this is due to a highly restrictive definition of the WHO,[11] or previous classifications,[15] since the *sine qua non* condition is the demonstration of cerebrospinal and/or systemic metastases, once there are no morphological criteria of malignancy. The time period between the initial diagnosis of adenoma to carcinoma is approximately 7 years, and the average survival, after confirmation of malignancy, is reported to be approximately 1.9 years,[16] or 1 year in two-thirds of the patients.[17] Since the suspicion of pituitary carcinoma is only confirmed by the existence of metastasis, this delays a more aggressive therapeutic approach, reducing its potential effectiveness. Due to the latency between initial diagnosis and appearance of metastases, it is often too late to treat the patient when spread appears. Earlier diagnosis and referral to specialized reference centres are fundamental to optimize short and long-term outcomes and prognosis in these patients.[6]

Differential diagnosis between an aggressive benign tumour and a malignant tumour in initial stage can be very difficult. The prediction of this type of tumours behaviour remains a challenge for both clinicians and pathologists; it seems necessary an early diagnosis, to allow an aggressive treatment of thosetumours, that do not reveal cytomorphologic features of malignancy *ab initio* and have worse prognosis. The aim of this study is to propose a new diagnostic strategy to orient prognosis and therapy of these tumours, based on a clinico-laboratorial and radio-histopathologic multiparameter system, as well as a simple diagnostic algorithm. This strategy derives from the retrospective analysis of the PA casuistic operated in the last 11 years at the largest hospital centre in Portugal.

Material and Methods

To validate the method, we applied this new clinicopathological classification retrospectively to patients diagnosed and operated by endonasal transsphenoidal via, with histological confirmation of PA, between

01/01/2004 and 31/12/2014, at Centro Hospitalar Lisboa Norte, consisting of Hospital Universitario de Santa Maria and Hospital Pulido Valente. The procedures followed were in accordance with the ethical standards of the responsible institutional committee and with the Helsinki Declaration of 1975, as revised in 2000. PA were classified according to the 2004 version of the WHO on tumours of endocrine organs.[11] The rate of recurrence in those patients followed up for at least 5 years has been evaluated.

We have designed a simplified, practical and easy to apply diagnostic algorithm for the distinction between "typical" adenoma (which we propose naming endocrine pituitary tumour -PET- of biological behaviour most likely benign) *vs* "atypical" adenoma (which we propose naming, based on their aggressiveness, PET of uncertain malignant potential or PET of biological behaviour most likely malignant).

This algorithm is based on a multiparameter system, none of which is an absolute criterion of malignancy if used alone, and uses a numeric score based on the association of a specified threshold for each parameter of malignancy. It includes criteria related to the cytological appearance, cellular proliferation index, expression of a tumour suppressor gene, invasion and tumour recurrence (Table 1).

For each tumour, the points for each parameter must be added to reach the total of score (*minimum* score: 0; *maximum* score: 10). Therefore:

0 to 3 points is consistent with: "typical" PA (according to WHO, 2004). We propose to call it: PET grade 1 (low-grade malignancy) / PET of biological behaviour most likely benign.

4 to 7 points is consistent with: "atypical" PA (according to WHO, 2004). We propose to call it: PET grade 2 (intermediate grade of malignancy) / PET borderline / PET of uncertain malignant potential.

8 to 10 points is consistent with: "atypical" PA (according to WHO, 2004). We propose to call it: PET grade 3 (high-grade malignancy) / PET of biological behaviour most likely malignant (carcinoma *in situ* or pre-metastatic).

In the presence of cerebrospinal and/or systemic metastases, the two ranking systems (WHO, 2004 and our proposal) call these tumours pituitary carcinoma.

For this, we define a few parameters, some of which are already used by the WHO in its classification for this type of tumours however without cut-off point referred.

We calculated the number of mitoses in representative high-power fields (HPF), according to the average per 10 HPF (HPF of 0.30 mm^2, x400 magnification).

The cell proliferation index (Ki67) was calculated as the percentage of positive nuclei within a minimum of 500 tumour cells in the areas of strongest immunostaining, analysed in optical microscope with x400 magnification. In equivocal cases, it was estimated with the help of an image processor software for immunohistochemical analysis, a method that compared with the performance of an experienced pathologist is matching 89.7% of cases.[18] As for p53, it is important that the dial intensity is moderate/intense, excluding the nuclei with weak dial (here the contribution of the software can be very valuable, by enabling to create a threshold of intensity).

Due to the occasional misdetection of p53 and the absence of avalidated prognostic cut-off value by WHO, this was calculated as for Ki67, considering as a positive a value ≥2, according to the proposal made by the German working group members on pituitary tumours.[19]

The tumour size and the extent of invasion are determined by MRI before surgery.[20] Tumours are classified as microadenomas (≤1 cm), macroadenomas (>1 and ≤4 cm) or giant adenomas (>4 cm). Following the WHO criteria,

Table 1: Proposed guide to assess malignant potential of PA (*minimum* score: 0; *maximum* score: 10).

Parameters:	Score		
	0	1	2
Number of mitoses	Absent or rare (<2 / 10 HPF)	Present but uncommon (2-5 / 10 HPF)	Present (and/or with atypical mitotic figures) (>5 / 10 HPF)
Ki67 (%)	≤3	>3 and ≤20	>20
p53 (%)	Negative	<2	≥2
Radiological classification	Grade 0-1	Grade 2-3	Grade 4
Tumour recurrence	No	Yes	Yes (2 or more)

HPF = High-power field (x400).
0-3 points: PET grade 1 (low-grade malignancy) / PET of biological behaviour most likely benign.
4-7 points: PET grade 2 (intermediate grade malignancy) / PET borderline / PET of uncertain malignant potential.
8-10 points: PET grade 3 (high-grade malignancy) / PET of biological behaviour most likely malignant (carcinoma *in situ* or pre-metastatic).

microadenomas are radiologically classified as grade 0 (intrasellar adenomas with normal appearance of the sella turcica) or grade 1 (intrasellar adenomas with enlargement of the sella turcica); macroadenomas are graded as grade 2 (tumours with diffuse sellar enlargement without bone erosion), grade 3 (tumours with focal bone erosion) and grade 4 (tumours with extensive bone erosion including the base of the skull and extrasellar structures).[11]

We define a postoperative recurrence during follow-up, as tumour recurrence with imaging studies for non-functioning as functioning adenomas, as well as clinical evidence of postsurgical disease by hormonal hypersecretion for functioning tumours.

In addition to the 5 parameters mentioned (mitotic index, Ki67 proliferative index, p53 immunostaining, tumour invasion, and recurrence), other criteria must be considered relevant, including cytomorphologic features, hormonal immunohistochemical subtypes, functionality of these tumours (clinical presentation), rapid progression of neurological signs or intra-operative observed invasion.

Cytological atypia must be graded with the x100 objective, according to the following degrees:

Without atypia/minimal atypia: round-to-ovoid uniform nuclei, with fine chromatin, inconspicuous nucleoli and a moderate quantity of cytoplasm.

Moderate atypia: large nuclei, with some pleomorphism, and open chromatin; recognizable nucleoli.

Marked atypia: pleomorphic nuclei, with rude chromatin, and large nucleoli.

Results

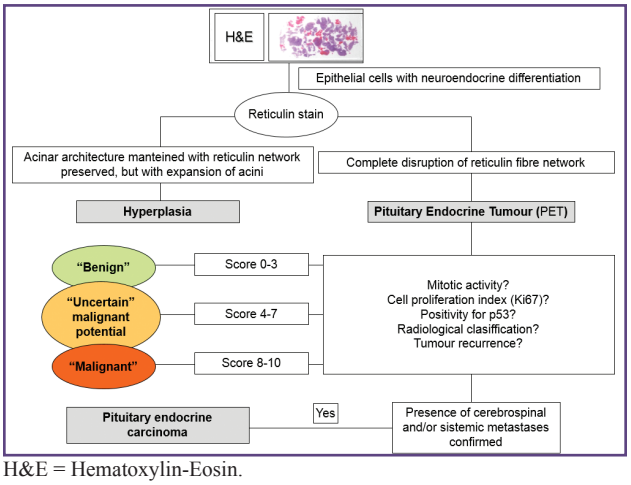
Of 243 operated patients, in 214 of them (88.1%) the tumour showed characteristics of "typical" adenoma and in 29 (11.9%) the characteristics of the tumour were of "atypical" adenoma. Then we apply our diagnostic algorithm to these tumours (Fig. 1).

Two hundred and sixteen cases (88.9%) were diagnosed as PET of biological behaviour most likely benign (2 of the tumours, that had been diagnosed as atypical with the WHO classification, both clinically "silent" ACTH-producing macroadenomas, presented with score 3 according to our classification system, having shown no recurrence of disease after 9 and 10 years of follow-up) (Fig. 2); 27 cases (10.7%) were diagnosed as PET of uncertain malignant potential (Fig. 3) and 1 case (0.4%) was diagnosed of PET of biological behaviour most likely malignant (Figs. 4 and 5) (Table 2).

In 129 of the 243 PA, the follow-up lasted more than 5 years; 113 of these 129 adenomas (87.6%) were diagnosed as PET of biological behaviour most likely benign and the remainder (16; 12.4%) as PET of uncertain malignant potential. Seven of the PET of biological behaviour most likely benign (7/113, 6.2%) had recurrence; of these, 5 were clinically non-secreting macroadenomas (71.4%), with positive immunostaining for prolactin in one case, gonadotrophin in 3 and TSH in the remaining; one case (microadenoma) presented clinically with Cushing's disease positive to ACTH, and there was a GH-secreting macroadenoma with acromegaly. Eleven of the PET of uncertain malignant potential (11/16, 68.8%) had recurrence; of these, 9 were clinically non-secreting macroadenomas (81.8%), with positive immunohistochemistry for prolactin in 2, ACTH in 2 ("silent"), gonadotrophin in 3 and TSH in 2; 2 cases (a microadenoma and a macroadenoma with pituitary apoplexy) presented clinically as Cushing's disease, positive for ACTH.

Discussion

Although there are verified differences between adenomas and carcinomas, the usual parameters cannot distinguish conclusively between benign and malignant pituitary neoplasms. With this work we intend to provide a more specific malignancy differentiating system, with a capacity to early identify cases of possible poor evolution, something that could be of great clinical utility. We believe that the proposed strategy for the diagnosis of PA, new and easy to use, can help firstly pathologists in the diagnostic decision, and secondly, clinicians choosing the best post-operative therapy, since that "uncertain" malignant potential tumours would require periodic monitoring, whereas those considered potentially "malignant", would require a more aggressive treatment. In any case, the multidisciplinary



H&E = Hematoxylin-Eosin.

Fig. 1: Simple algorithm for the primary proliferation of adenopituitary cells.

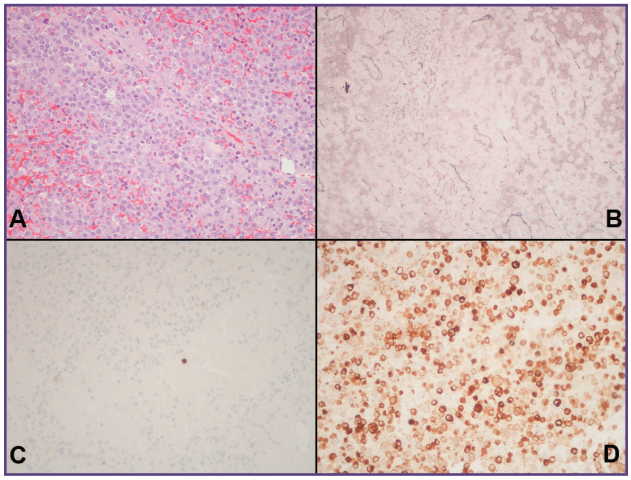


Fig. 2: Photomicrographs of a PET of biological behaviour most likely benign positive for GH. A) There is a proliferation of monomorphic cells with round-to-ovoid nuclei and moderate amounts of eosinophilic cytoplasm (H&E x200). B) A reticulin stain demonstrates effacement of the usual adenohypophysis acinar architecture (Gomori Reticulinx 200). C) Cell proliferation index is low (<1%, Ki67 x200). D) Tumour shows cytoplasmic immunoreactivity for GH (GH x200).

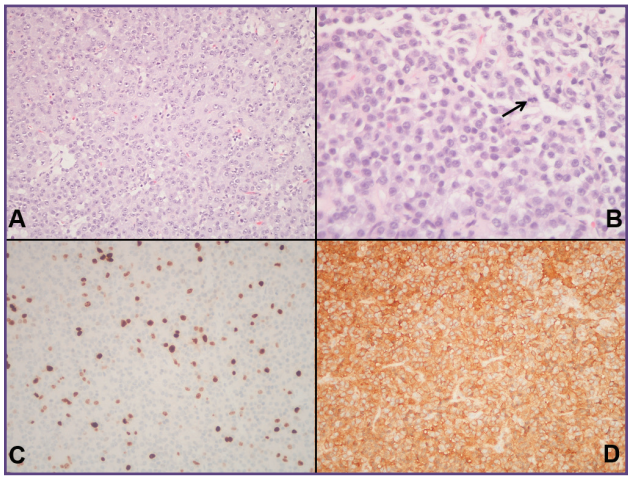


Fig. 3: Photomicrographs of a PET of uncertain malignant potential positive for GH. A) This is a moderate to densely cellular tumour, composed of large and occasionally pleomorphic cells, prominent nucleoli and a moderate quantity of pale eosinophilic cytoplasm (H&E x200). B) Scattered mitotic figures are seen (arrow) (H&E x400). C) The tumour shows high proliferative index (5%, Ki67 x200) and diffuse cytoplasmic immunoreactivity for GH (D - GH x200).

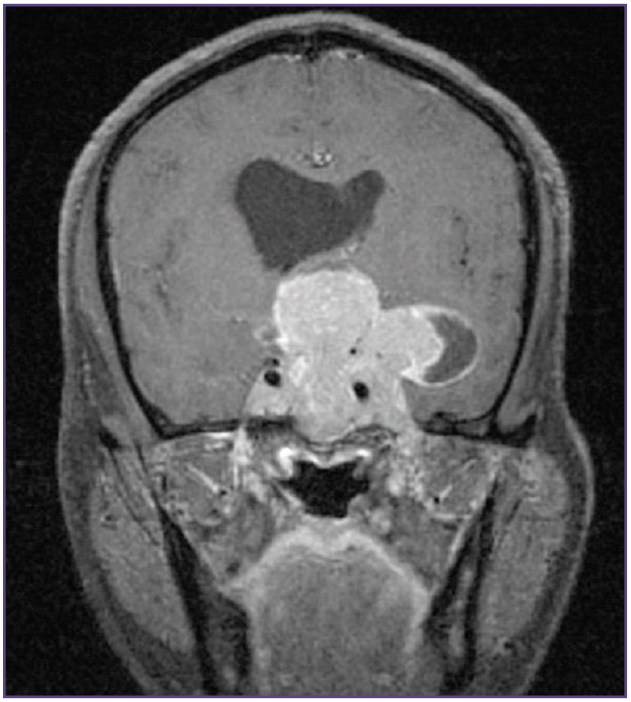


Fig. 4: Preoperative post contrast coronal T1 MRI, obtained in a patient with an "atypical macroadenoma" classified as PET of biological behaviour most likely malignant. Note the high propensity for bilateral invasion to the cavernous sinus, with compression of the aqueduct and incipient hydrocephalus.

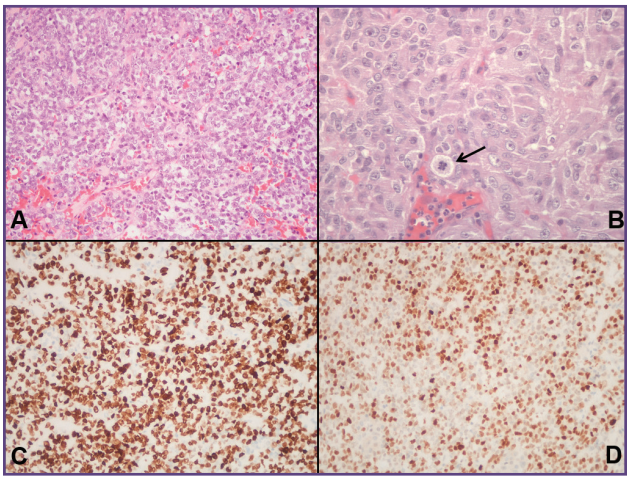


Fig. 5: Photomicrographs of a PET of biological behaviour most likely malignant that showed no immunoreactivity for any hormone. A) It is a densely cellular tumour composed of large and pleomorphic cells, prominent nucleoli and moderate amounts of eosinophilic cytoplasm (H&E x200). B) Abundant and sometimes atypical mitotic figures can be observed (arrow) (H&E x400). C) The tumour shows high proliferative index (39%, Ki67 x200) and extensive nuclear immunoreactivity for p53 (D - p53 x200).

Table 2: Comparative diagnostic study.

	No. of patients (%)			
Patients n = 243 (100 %)	According to the WHO (2004)	Typical adenoma 214 (88.1)	Atypical adenoma 29 (11.9)	
	According to the new classification proposal	PET "benign" (grade 1) 216 (88.9)	PET of "uncertain" malignant potential (grade 2) 26 (10.7)	PET "malignant" (grade 3) 1 (0.4)

PET = Pituitary Endocrine Tumour.

consensus on the best therapeutic decision, also requires a personalized medicine for each patient.

Mitoses are rare in adenomas and particularly in microadenomas, where they were found in only 3.9% of invasive adenomas in one of the largest studies to date.[21] Mitosis can be seen in 21.4% of invasive adenomas and 66.7% of carcinomas.[12] It is not established in the WHO classification the number of mitoses that favours the diagnosis of atypical adenoma, being subjectively referred "(…) *an elevated mitotic index* (…)". A recent study conducted in Germany suggests a higher number than 2 per 10 HPF to consider invasive a PA, with a sensitivity of 0.90 and a specificity of 0.74, being one of the data that will require future consensus.[19]

The use of immunohistochemical studies with Ki67 and p53 for PA has been controversial. Ki67 is a commonly examined antigen in PA, as it can contribute to define a group of adenomas with locally more aggressive behaviour. Increased levels of this antigen are correlated with growth speed, invasion and tumour recurrence.[22] In 1996, the study of Thapar et al. showed that the increase of Ki67 above of 3% is significant to differentiate invasive from non-invasive PA, and this threshold was accepted by the WHO. Their studies reported a Ki67 proliferative index of 1.4%, 4.7% and 11.9% in the non-invasive adenomas, invasive adenomas and carcinomas, respectively. The 3% threshold was used to distinguish non-invasive adenomas of invasive adenomas with 97% specificity and 73% sensitivity.[23] However, studies of cell proliferation with Ki67, unfortunately did not show a consistent correlation with invasiveness or tumour recurrence,[24,25] although three recent publications[26-28] support the concept that only a Ki67 proliferative index higher than 20-30%, suggests the presence of an *in situ* pituitary carcinoma,[29] or a pre-metastatic pituitary carcinoma in "sellar phase";[30] this would be independent of the tumour size and the presence or absence of local invasion.

P53 immunoreactivity has been found in all pituitary carcinomas.[10] It is not established in the WHO classification the percentage of positive nuclei and intensity of immunohistochemical staining for tumour suppressor gene p53, also being subjectively indicated "(…) *as well as extensive nuclear staining for p53 immunoreactivity*". A recent study recommends a cut-off value in the definition of this type of tumours in upcoming editions, suggesting a ≥2% cut-off .[19]

In spite of this, routine use of Ki67 and p53 immunohistochemistry is not a common practice in many experienced laboratories, because it is not clear for the clinical team that treats the patient, how to evaluate the information that an adenoma is histologically "atypical". In addition, factors such as size and tumour extension at the time of surgery may seem more relevant than the cellular proliferation. Therefore, the clinical usefulness of this category to identify eventually metastatic tumours is yet to be establish.

Invasiveness is defined as the extension to the bone of the sellar floor, cavernous sinus and/or sellar diaphragm,[21] according to the assessment in preoperative neuroimaging studies. Although some studies have shown that invasion itself does not correlate with recurrence or with a worse prognosis, the majority of patients who die because of tumours of the pituitary gland have invasive adenomas.[31] Some experts have pointed out that the WHO classification of 2004 did not take into account the state of the invasive tumour.[22]

To date, there are hardly any studies that indicate that "typical" PA has lower rates of surgical remission, or that the PA called "atypical" shows higher rates of recurrence.[22] In our study, while 6.2% of PET of biological behaviour most likely benign presented recurrence, 68.8% of those which we classify as being of uncertain malignant potential did (the probability of postsurgical tumour recurrence in a follow-up longer than 5 years is eleven times higher; $p<0.0001$). In the recurrent tumours, we also observed an increase in the cell proliferation index (Ki67), 2.73% for PET of uncertain malignant potential compared to 0.29% for PET of biological behaviour most likely benign.

The standard morphological characteristics associated with malignancy, including hypercellularity, nuclear and cellular pleomorphism, increased mitotic activity, necrosis and dural/bone invasion, are commonly present in

carcinoma, although, as in other endocrine organs, they are not necessarily diagnostic.

Some PA are "intrinsically" aggressive (such as prolactinomas in postmenopausal women and those that occur in young men, sparsely granulated GH-producing adenomas or "silent" ACTH adenomas).The majority of pituitary carcinomas are hormonally active, representing prolactinomas and ACTH-secreting tumours two thirds of the same,[17] although any histologic type and secretory pattern it has been described. Recent studies reveal that 91% of prolactinomas are invasive and 55% show a Ki67>3%. Other proliferative adenomas are gonadotroph/null and corticotroph.[25,32] Pituitary tumours in patients with multiple endocrine neoplasia syndrome type 1 (MEN1) tend to be larger, invasive and symptomatic, although differences between these tumours and the rest of PA has not been demonstrated.

Conclusion

Early identification of aggressive endocrine tumours would allow the implementation of an intensive treatment that could prevent the recurrence or metastasis. Similarly to other endocrine tumours with problems in defining the histological criteria of malignancy, we present here our proposal for clinicopathological classification, based on a multiparameter grading system, which may incorporate additional clinical and pathological factors. This clinicopathological classification, that evaluates and categorizes the endocrine pituitary tumours in degrees or potential for malignancy, presents advantages such as: 1) assign a prognostic value in predicting a postoperative evolution free of disease or recurrence for each type of tumour; it is more precise than the current system of the WHO and has been shown to have relationship with the biological behaviour of the tumour; 2) is an objective, practical, easy to use and reproducible classification system, with potential to decrease the interobserver variability and, 3) identify the tumours that require a more aggressive treatment, as well as those indolent that might be more consensual.

The importance of early identify potential immunohistochemical and molecular markers of invasion and malignancy, enable us to develop therapeutic aimed at improving the prognosis of affected patients. Finally, it would be desirable to reassess the definition, classification and criteria of malignancy that should be applied to pituitary neoplasms, specifically to the PA called atypical.

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Competing interests

None declared.

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