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Pituitary neuroendocrine tumors;
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SF1

The immunohistochemistry (IHC) characterization of pituitary transcription factors (PTFs) ication of three different adenohypophyseal l lines, ication system of the World Health Organizatio WHO) erturns the concept of the adenoma as solely a horm e pro- sed on their cell lineage. The aim of the study was to p vide a C expression of hypophyseal hormones with potential use n diag- an improved classification of pituitary adenomas. Our sample cluded viously classified based on hormonal subtypes by IHC (former 200 WHO d after the IHC quantification of PIT1, TPIT, and SF1 expression, und WHO ns. We assessed the correlation between expression of PTFs and the class cation HC and correlated clinicopathological profiles based on PTFs. The IHC s udy of reclassification of 82% of tumors that were negative for all pituitary hormon , with cases for SF1 (reclassified as gonadotroph tumors), 1 positive case for TPIT (recl ssified ucotroph tumor), and 4 positive cases for PIT1. Using SF1 enabled detection of a sub tantial

☆ Disclosure

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portion of gonadotroph tumors, reducing the estimated prevalence of null cell tumors to less than 5%, and identification of plurihormonal pituitary neuroendocrine tumors with PIT1-SF1 coexpression and hormone-negative PIT1s, a group in which we did not observe differences in the clinical behavior compared with the rest of the tumors of the same cell lineage.Our results suggest that applying a diagnostic algorithm based on the study of PTFs could contribute to improving the classification of pituitary adenomas. By adding TPIT assessment, we propose a two-step algorithm, with hypophyseal hormones being used in a selective modality, depending on initial results.

1.

In recent years, new proposals on the classification of pituitary tumors have been published, with most relevant updates presented by the World Health Organization (WHO) in 2017 [1]. Significant changes affecting the diagnosis of pituitary adenomas (PAs) were introduced, and the use of lineage-restricted pituitary transcription factors (PTFs) was endorsed. The revised classification suggests that there may be special morphological subtypes of PAs that entail higher risks owing to their clinical aggressiveness, although the impact of these subtypes as predictors of treatment response is not universally accepted [2]. Meanwhile, panels of experts have also proposed replacing the term PA for pituitary neuroendocrine tumor (PitNET) [2–4], taking into account the spectrum of variability of behavior of these tumors. Others have questioned this denomination [5].

All these considerations need to be translated in a most practical way to routine clinical practice, providing surgical pathologists with validated and standardized algorithms and recommendations for accurate diagnosis of PitNETs. At present, there are antibodies available for formalin-fixed, paraffin-embedded tissue specimens that allow the use of transcription factors in routine diagnosis [6–8]. Although their assessment is conditioned by lack of validation [4], some recommendations on their application exist, given the importance of making an accurate diagnosis [6]. A diagnostic algorithm, based on immunohistochemistry (IHC) expression of hypophyseal hormones, enables PitNETs to be classified by type and subtype [4], with PTFs being used in a second step to divide PitNETs into three different lineages. In this study, we present the results of an alternative approach based on the initial study by IHC expression of PTFs in a large series of PitNETs with clinicopathological correlation, providing a practical, simple approach with potential use in diagnostic practice and contributing to an improved classification of PAs.

2.

We retrospectively studied all cases with a pathologically confirmed diagnosis of PitNET, in patients who underwent surgery from January 2009 to January 2020 in Hospital Universitario de Alicante (Spain). Samples were obtained from the hospital's biobank collection after informed consent was granted and approval was received from the bioethics committee. All included cases had been classified following 2004 WHO criteria, by applying a panel of antibodies including pituitary hormones: growth hormone (GH; polyclonal rabbit antihuman growth hormone; Dako-Agilent), ACTH (monoclonal mouse antiadrenocorticotropin; Monoclonal Mouse Anti-Adrenocorticotropin (ACTH), Clone 02A3, Dako-Agilent), follicle-stimulating hormone (FSH; monoclonalmouse antieFSH, clone C10; Dako-Agilent), and luteinizing hormone (LH; monoclonal mouse anti-luteinizinghormone, clone C93; Dako-Agilent) North America, Inc. 6392, vía real, Carpintería, California, 93013 USA). Prolactin (PRL; monoclonal Prolactin B109.1; Santa Cruz Biotechnology) 2145 Delaware Avenue, Santa Cruz, CA. 95060,U.S.A.

Samples collected after clinical relapse were excluded, as well as double PitNETs (3 cases) and biopsies with scarce material, necrosis, or poor tissue conservation. The final cohort included 146 patients.

Electronic health records were reviewed to obtain all clinical data and results of the hormonal IHC and cell proliferation assay; cytokeratin staining came from the Access database of the Pathology Service. Positivity for hormone IHC analysis was defined as expression of at least 5% of tumor cells.

2.1.

IHC was performed to study PIT1 lineage (PIT1, polyclonal, Thermo Fisher)Carrer Roll de Colomer, 21, 46138 Rafelbunyol, Valencia, TPIT lineage (TPIT, monoclonal CL6251, Abcam), and SF1 lineage (SF1, monoclonalEPR19744, Abcam) Discovery Drive Cambridge Biomedical Campus, Cambridge CB2 0AX, Reino Unido expression on tissue microarrays, which were previously constructed with the selected cases, taking two punches with a diameter of 1 mm for each tumor on blocks of paraffin tissue and placing them on a recipient block using a Beecher Instruments tissue arrayer. Each block included

20 cases (two cores per case), plus two controls. For PTF, the detection system used was the ultraView Universal DAB Detection Kit on a BenchMark ULTRA Instrument (Ventana). Ventana Medical Systems Inc, 1910 E Innovation Park Dr, Oro Valley AZ 85755-1962. Staining quantification was performed by three observers under a multihead microscope. The results are expressed as percentage and intensity of positive cells. Staining equal or superior to 5% of tumor cells was considered positive (similar to hormonal IHC criteria), excluding areas adjacent to the anterior pituitary.

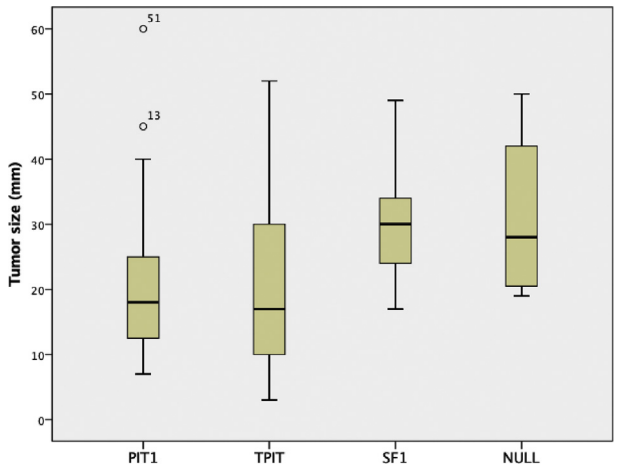
2.2.

Evaluation of Ki-67 (monoclonal 30-9, Ventana) proliferative activity was performed in complete sections on two hot spots, quantifying at each spot at least 500 cells and considering any intensity of nuclear staining to be positive. The results are expressed as a percentage, considering Ki-67 proliferation of 3% or more to be high. For the study of cytokeratins (monoclonal rabbit antihuman cytokeratin 8/18, clone EP17/EP30; Dako-Agilent), we examined the pattern of the stain (dot-like or perinuclear), defining tumors as positive when at least 5% of the cells were stained. IHC assays, with exception of PTFs, were performed as per a routine staining method using a streptavidin-biotin system (LSAB+, ChemMate Detection Kit 5301 Stevens Creek Blvd. Santa Clara, CA, 95051, United States) by using a Dako-OMNIS automated staining platform (Dako-Agilent).

All data obtained were added to the Access database, which was exported and analyzed using the SPSS statistical program (version 23) IBM Corporation New Orchard Road Armonk, NY 10504. The results were compared in contingency tables (chi-square and Fisher's test), and correlation between quantitative variables was analyzed using Spearman's rho test. To analyze the medians and box plots, the Kruskal-Wallis H (nonparametric means for more than two groups) test was used.

3.

The mean age of the patients was 53 years (range = 14−84), and 81 of 146 (55%) patients were men. The clinical manifestation was acromegaly in 29 patients (20%). Ten (7%) were clinically diagnosed with prolactinoma—all with a locally aggressive course or resistant to treatment with dopamine receptor agonists, and another 10 patients (7%) had Cushing disease. One (0.7%) showed clinical signs of TSH-secreting tumor with biochemical hyperthyroidism, and 96 (66%) had symptoms of local tumor growth, mainly headache and visual impairment, with no signs derived from hormone production (nonfunctioning tumors). In terms of radiological presentation, 131 (90%) cases were macrotumors, 73.3% of which were nonfunctioning, and 14 (10%) cases were microtumors, all of which were functioning. Tumor size on nuclear magnetic resonance (NMR) ranged from 3 mm to 60 mm (median = 25 mm). Infiltrative growth to the sinus was identified in 74 (51%) cases.



. 1 Box plot with tumor size based on the subtype of pituitary transcription factors ($P < 0.001$).

3.1.

Hematoxylin and eosin staining showed that 21 cases had a predominance of acidophils; 83, chromophobes; 29, mixed acidophil-chromophobe; and 13, basophils. The main architectural pattern was diffuse/sinusoidal (60%), followed by sinusoidal with pseudorosettes/papillae (40%).

3.2.

Forty-eight cases (32.9%) were positive for PIT1 (mean positive cells: 97%, range = 30−100%); 22 (15.1%) were positive for TPIT (mean = 68%, range 10−100%); and 70 (48.6%) were positive for SF1 (mean = 67%, range = 6−100%). Six PitNETs were negative for all PTFs. Three of the PIT1-positive PitNETs showed focal expression of SF1 (mean = 15%, range = 10−21%; Fig. 1). Table 1 shows the correlation between the clinical diagnosis and the expression of PTFs, and Table 2 shows the correlation between the expression of PTFs and pituitary hormones.

3.3.

IHC for cytokeratin 8/18 was positive (5%) in 115 (78.8%) of the samples, with 76.9% showing a perinuclear staining pattern, 17.1% showing a dot-like pattern, and 6% showing a mixed perinuclear-dot pattern. Most of the tumors (96%) that were negative for cytokeratin were positive for SF1 or classified as null cell.

The Ki-67 proliferation fraction was quantified in 144 PitNET cases: 14 (10%) showed high proliferative activity (3%). The group with the largest proportion of high-Ki-67

1 Clinical presentation and expression of pituitary transcription factors (PTFs) ($P < 0.001$).

Clinical diagnosis	n (%)	Expression of PTFs				
		PIT1	TPIT	SF1	PIT1/SF1	NULL
Acromegaly	29 (19.0)	26	0	0	3	0
Hyperprolactinemia	10 (6.8)	10	0	0	0	0
Cushing disease	10 (6.8)	0	10	0	0	0
Hyperthyroidism	1 (0.7)	1	0	0	0	0
Nonfunctioning	96 (65.8)	8	12	70	0	6
Total	146	45	22	70	3	6

cases was of TPIT lineage, followed by PIT1, and within the latter, lactotroph tumors (LTs; n = 4), albeit the differences, were not statistically significant.

After defining the groups based on PTFs (PIT1 and PIT1/SF1 PitNETs were classified into the PIT1 group), we tested the association with gender. There was a predominance of women in the PIT1 group (60%) and of men in the TPIT (55%) and SF1 (66%) groups ($P = 0.050$). The statistical analysis (Kruskall-Wallis H) showed significant differences in median ages and in tumor size between the groups ($P < 0.001$) (Fig. 1). Patients with SF1 and null cell tumors were older and had larger tumors, followed by the TPIT group.

3.4.

3.4.1.

3.4.1.1. Somatotroph tumors. Thirty cases were positive for GH and classified as somatotroph tumors (STs). The mean age in this group was 46 years (range = 24–71), and 70% were women. Except for one case that was classified as nonfunctioning, all were clinically diagnosed with acromegaly. As per NMR, 25 were macrotumors (83%), with a mean size of 20 mm (range = 7–27), and 12 (40%) showed invasion to the sinus. Microscopically, 11 were composed of eosinophilic cells, 14 were composed of chromophobic cells, and 5 were composed of mixed eosinophilic and chromophobic cells.

Nine tumors that presented GH with high-density staining and eosinophilic morphology were classified as densely granulated ST (DGST). Seven of these showed a perinuclear cytokeratin pattern, and two cases showed a dot-like pattern (DGST of the intermediate type). Nine tumors with low-density GH staining, indicative of chromophobic cells and with a dot-like cytokeratin pattern, were classified as sparsely granulated ST (SGST). Twelve tumors showed coexpression of PRL: six that were densely granulated and had perinuclear cytokeratin patterns were classified as mammosomatotroph tumors, while six that were sparsely granulated and showed a dot-like cytokeratin pattern were classified as mixed ST-LT.

Three more were positive for SF1 (Fig. 2): one DGST, one SGST, and one mammosomatotroph tumor, all of them with PIT1 positivity higher than 95%. SF1 positivity ranged from 12% to 25%. Two tumors—one DGST and one SGST—showed a high Ki-67 index (3%). The test for association and comparison of medians between the four subgroups, as per granular intensity or pure versus mixed STs and by age, sex, tumor size, invasiveness, and proliferative activity, did not show statistically significant differences.

3.4.1.2. Lactotroph tumors. Eleven cases were classified as LT, all of which were positive for PRL. The patients' mean age was 37 years (range = 14–72), and there were six women (54.5%). In 9 cases (82%), clinical presentation was concordant with prolactinoma, whereas the remaining tumors were silent. Eight (73%) were macrotumors, with a mean size of 24 mm (range = 9–60), and five (45%) presented invasion to the sinus. Most (82%) were

2 Correlation between the expression of pituitary transcription factors (PTFs) and the classification by hormonal IHC ($P < 0.001$).

PTF expression	n (%)	IHC hormonal subtype					
		ST	LT	CT	TT	GT	Negative
PIT1	45 (38.8)	27	11	0	3	0	4
PIT1-SF1	3 (2.1)	3	0	0	0	0	0
TPIT	22 (15.1)	0	0	21	0	0	1
SF1	70 (47.9)	0	0	0	0	49	21
Null PTF	6 (4.1)	0	0	0	0	0	6
Total	146	30	11	21	3	49	32

Abbreviations: ST, somatotroph tumor; LT, lactotroph tumor; CT, corticotroph tumor, TT, thyrotroph tumor; GT, gonadotroph tumor; IHC, immunohistochemistry.

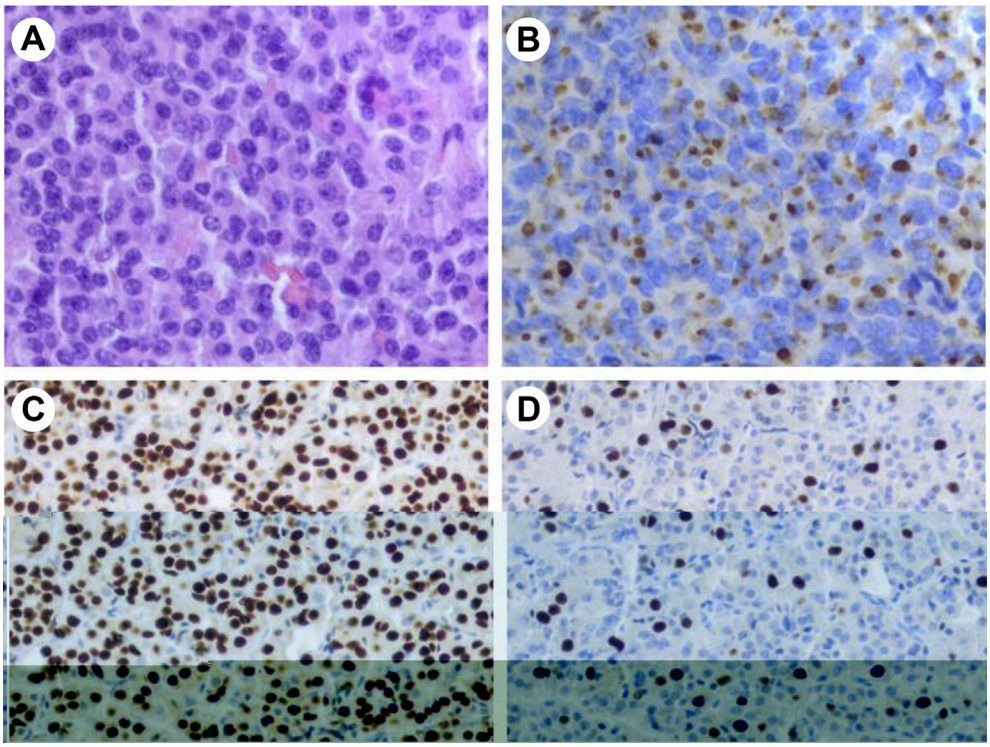


Fig. 2 Sparsely granulated growth hormone tumor (SGST) with coexpression of PIT1 and SF1. A, Tumor formed by monomorph proliferation of small chromophobic cells (H&E, ×200). B, Inmunostaining with cytokeratin 8/18 showing cytoplasmic juxtanuclear reactivity (fibrous bodies) (×200). C, Inmunostaining nuclear positivity with PIT1 (95%) (×200). D, Positivity in dispersed cells (12%) for SF1 (×200). H&E, hematoxylin and eosin; SGST, sparsely granulated somatotroph tumor.

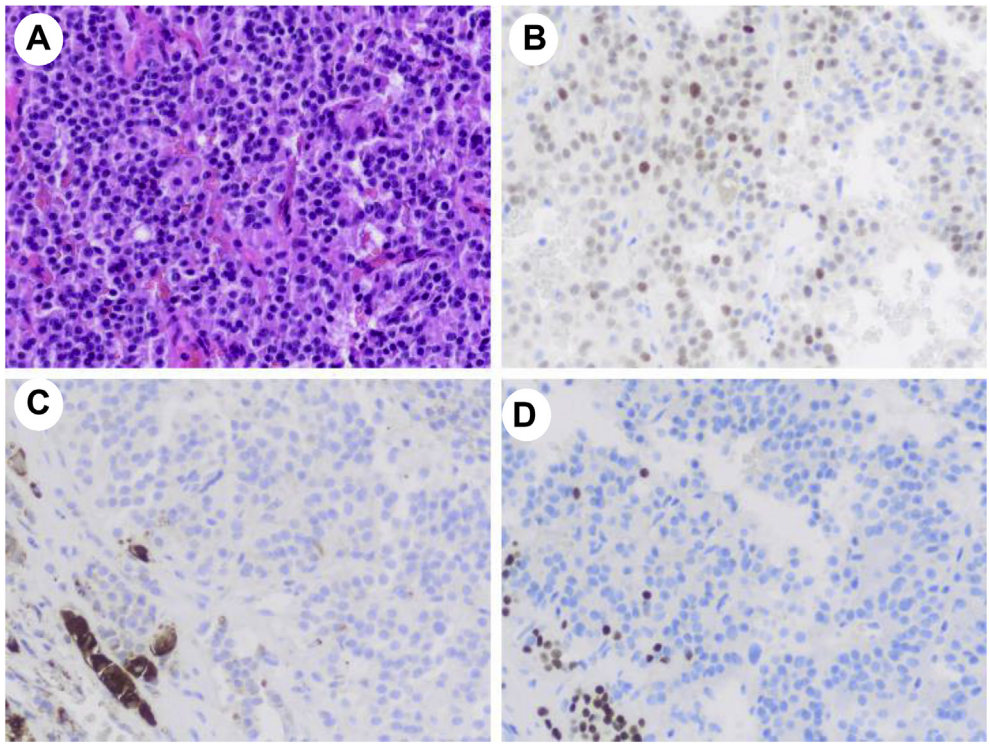
chromophobes. On IHC, one case showed dense immunostaining for PRL, concordant with a densely granulated LT subtype. The rest (n = 10) showed a paranuclear (Golgi-type) pattern and were classified as sparsely granulated LTs. Two (18%) showed positivity for GH, eosinophilic cellularity, nuclear pleomorphism, and dot-like cytokeratin; these were classified as acidophil stem cell tumors. Two others were negative for cytokeratins, and four (36%) presented Ki-67 values higher than 3% (high proliferative activity).

3.4.1.3. Thyrotroph tumors. Three patients (two men) had thyrotroph tumor (TT). Their mean age was 54 years (range = 42–76). One had clinical signs of TSH-secreting tumor with primary hyperthyroidism, and two were silent (Table 2). Two cases were macrotumors, one with invasion to the sinus. Staining was indicative of chromophobes or weak eosinophils, with abundant fibrous stroma. In all three cases, immunostaining for PIT1 and TSH was positive, and one showed focal coexpression of LH, albeit SF1 was negative. The three tumors were positive for cytokeratins and showed low Ki-67 indexes.

3.4.1.4. PIT1 tumors without hormone expression. Four (12.5%) of the 32 PitNETs defined as null cell by hormonal IHC were positive for PIT1. One patient, diagnosed with prolactinoma three years before the surgery and treated with cabergoline, was negative for PRL as per IHC. The three remaining PitNETs were silent. The mean age of these patients, all of whom were men, was 50 years (range = 25–66). All the tumors were invasive macrotumors, with a mean size of 22 mm (range = 16–25). Microscopically, two had a predominance of chromophobes and one was eosinophilic. The three were positive for cytokeratin, with a perinuclear pattern, and showed a low Ki-67 index.

3.4.2.

Twenty-two were TPIT positive. The patients' mean age was 49 years (range = 19–68), and 12 (55%) were men. Ten were diagnosed with Cushing disease, whereas 12 (55%) had silent tumors. Most of the patients with Cushing disease were women (70%), whereas 75% of the silent corticotroph tumors (CTs) were found in men ($P = 0.046$). Six cases were microtumors (27%) and 16 were macrotumors, with signs of invasiveness in 9 cases. The mean tumor size was 22 mm (range = 3–52). All the microtumors manifested as Cushing disease, and all the silent ones were macrotumors ($P = 0.006$). Microscopic staining indicated that 9 (41%) had a predominance of basophils, whereas the rest had a predominance of either chromophobes (32%) or mixed basophil-chromophobes (27%). Clinical signs were concordant with



. 3 TPIT-positive pituitary neuroendocrine tumor. A, Chromophobic cells with H&E staining (×100). B, Tumor cells showing inmunohistochemical staining for TPIT with variable intensity (TPIT, ×100). C, ACTH is negative in tumor cells, but shows positivity in scattered cells of nontumorous adenohypophysis (ACTH, ×100). D, Pit-1 is negative in tumor cells and positive in peripheral adenohypophysis and entrapped nontumorous cells (Pit1, ×200). ACTH, Adrenocorticotropin hormonr; H&E, hematoxylin and eosin.

Cushing disease in 68% of cases with a predominance of basophils, compared with 33% of cases with a predominance of chromophobes ($P = 0.2$). Fifteen presented solid or trabecular architecture, whereas the other seven had a pseudorosette pattern. Twenty-one were ACTH positive, with an average of 86% of stained cells (range = 10−100%), without differences between functioning and nonfunctioning tumors. One was classified as null cell tumor as per hormonal IHC (Fig. 3). Of those that were positive, nine were densely granulated (DG, six with Cushing disease), nine were sparsely granulated (SG, two functioning), and three were Crooke's cell CTs (two functioning). Four of the five microtumors were DG CTs. The three Crooke's cell CTs and the ACTH-negative TPIT were invasive macrotumors. Four CTs presented a Ki-67 index higher than 3%; all were noninvasive and DG (three microtumors and one macrotumor). All the TPIT tumors were positive for cytokeratin 8/18, with diffuse perinuclear cytoplasmic positivity.

3.4.3.

Seventy-three were positive for SF1 (cellular positivity range = 10−100%, mean = 70%). As mentioned previously, three cases coexpressed PIT1. The other 70 expressed only SF1 and were all nonfunctioning. The patients' mean age was 60 years (range = 31−84), and two-thirds (66%) of the patients were men. All cases were macrotumors and had a mean size of 29 mm (range = 17−49), with 39 (56%) showing invasion to the sinus.

Microscopically, 46 showed a predominance of chromophobes (66%), and 24 showed a predominance of eosinophilic cells, attributable to oncocytic change. In 47 (67%) cases, there was a predominant pseudorosette architecture, whereas in the rest, the pattern was diffuse or trabecular. With hormonal IHC, 24 (34%) cases expressed LH; 9 (13%), FSH; and 16 (23%), LH and FSH. Twenty-one (30%) were negative for gonadotropins (null as per hormonal IHC). Despite this result, a high correlation between positivity for LH/FSH and SF1 expression was observed (r = 0.609, $P < 0.001$, Spearman's rho). Four cases presented low levels of positivity for TSH (<20%), with negativity for PIT1. In 28 (40%) cases, cytokeratin staining was negative. In all positive cases, the staining pattern was perinuclear/diffuse. Four (6%) cases had a Ki-67 index higher than 3%.

3.4.4.

Of the 32 cases classified as null cell by hormonal IHC, just 6 (4.1% of the total sample) met the current criteria for this diagnosis (1), whereas the rest were classified as gonadotroph tumor (n = 19), silent PIT1 (n = 6), or silent CTs (n = 1). The six cases of true null cell tumors were

nonfunctioning; the patients' mean age was 62 years (range = 54−72), and there were three women and three men. All were macrotumors, with a mean size of 28 mm (range = 19−50), and four met the criteria for invasiveness. Microscopically, the six presented pseudorosette architecture; five showed a predominance of chromophobes, and one had spots with eosinophilic cells (oncocytic change). One (17%) was negative for cytokeratins, and all presented low proliferative activity as measured using the Ki-67 index.

4.

The uptake of PTF assessment in clinical practice, as recommended in the most recent WHO classification [1], enables the identification of the three hypophyseal cell lines, which, used in conjunction with hormonal IHC, allows substantial improvements in the diagnostic precision for PitNETs [2,4,6−12]. The clinical importance of adequately classifying PitNETs resides in the variations of their biological behavior and differential response to medical treatments, as well as the avoidance of erroneously diagnosing other neoplasms [6]. An important limitation, however, is the absence of standardized procedures, validated antibodies, dilutions, and cutoffs, which hinders the performance of comparative studies of the different PitNET subtypes. Adequate optimization of the techniques and preanalytic parameters are necessary to guarantee the quality of the results [6]. As an alternative, some authors have proposed studying PTFs via molecular techniques, with good results [13]. To date, few studies have assessed PTFs in case series, and some of these are limited to two PTFs [10] or to nonfunctioning tumors [7,11,12]. The largest published series so far, by Mete et al. [8], was in 1055 PitNETs, comprising 42.5% of gonadotroph tumors (96% positive for SF1), 29.9% of PIT1-positive tumors, 17.1% of CTs, and 4.5% of null cell tumors. Their results are highly concordant with those obtained in the present study (49.7% positive for SF1, 32.9% positive for PIT1, 15.1% positive for TPIT, and 4.1% null cell), although our sample showed a higher proportion in the SF1 group and a lower proportion of CTs/TPIT tumors; the prevalence of null cell tumors was practically the same. The study of PTFs enables identification of the null tumors that must be differentiated from the LH/FSH-negative gonadotroph tumors because they present different characteristics and behavior [14]. As with most surgical series, most of our patients (62.2%) presented nonfunctioning macrotumors, with an average age of about 52 years [1,7,8,10,15−18].

The assessment of the PTFs enabled the identification of the cell lineage in 96% of the PitNETs. The most heterogeneous group was the PIT1-positive PitNET, comprising STs, LTs, and TTs, with a 100% sensitivity for these subtypes. Three cases presented a coexpression of SF1, classifiable as unusual plurihormonal PitNET because they have two cell lineages. These cases were included as variants within the PIT1 group. In addition, four of the cases initially classified as null cell tumors by hormonal IHC were reclassified as pure PIT1 PitNETs. As per these results, we propose a diagnostic algorithm based on the immunohistochemical study of the PTFs at the first level (Fig. 4).

Most PIT1 tumors manifest clinically as acromegaly (1, 8, 19), which is consistent with our results (60.4%). All the patients with acromegaly presented STs, mammosomatotroph tumors, or mixed ST-mammosomatotroph tumor. With cytokeratin 8/18, we observed a slight predominance of DG compared with SG (52% vs 48%). Although some studies suggest that SG tumors are more aggressive [2,8,19], we did not observe differences with regard to size, invasiveness, or Ki-67 proliferation in DGST, mammosomatotroph tumors, SGST, and mixed ST-LT, although the small number of cases limits the statistical power of the results.

Of the 11 cases of LT, most were SG, which is in concordance with the literature [1,8,18]. The prevalence of acidophil stem cell tumors within the LTs varies widely by series (2−24%) [8,18]. Our results (18%) are nearer to the upper limit of this range. Acidophil stem cell tumors, initially identified by electronic microscopy [20], present as prolactinoma, but with several peculiar morphological features, such as oncocytic change, a higher degree of nuclear pleomorphism, microvacuoles corresponding to megamitochondria, and expression of PRL and often GH [1]. These morphological criteria may not be very precise, which would explain the variations in estimates of their prevalence. As in previous series, all the LTs were PIT1 positive (sensitivity = 100%) [2,8]. The behavior of these tumors is variable: they can usually be controlled with medical treatment, but at times (especially in men), their behavior is locally aggressive and their response to dopaminergic agonists is poor [21].

TTs are the least prevalent group of PitNETs in general [1,2,8,18,22−24] and in the PIT1-positive group [2,8]. In our series, they represent 2% of the total, a result which is more similar to the German registry [18] than to series in the study by Mete et al.' [8]. Within the PIT1 group, 6% were TTs. This type of PitNET can be functioning, producing hyperthyroidism, or silent, and they generally present a low Ki-67 index [23], all data being concordant with the three cases in our series. One of the TTs showed focal expression of LH but was SF1 negative. In the series of Căpraru et al. [23], they described 6 plurihormonal cases of a total of 20 cases of TT. Most patients see a complete remission after surgery [22−24]. However, it is important to rule out the possibility of cross-reactions between antibodies in these cases, especially between LH, FSH, and TSH, and only antibodies should be applied against the beta fraction, avoiding cross-reactions against the common alpha component. The application of PTFs should contribute to a better classification of cell lines in case of doubt [6]. Most published series based on PTFs find a

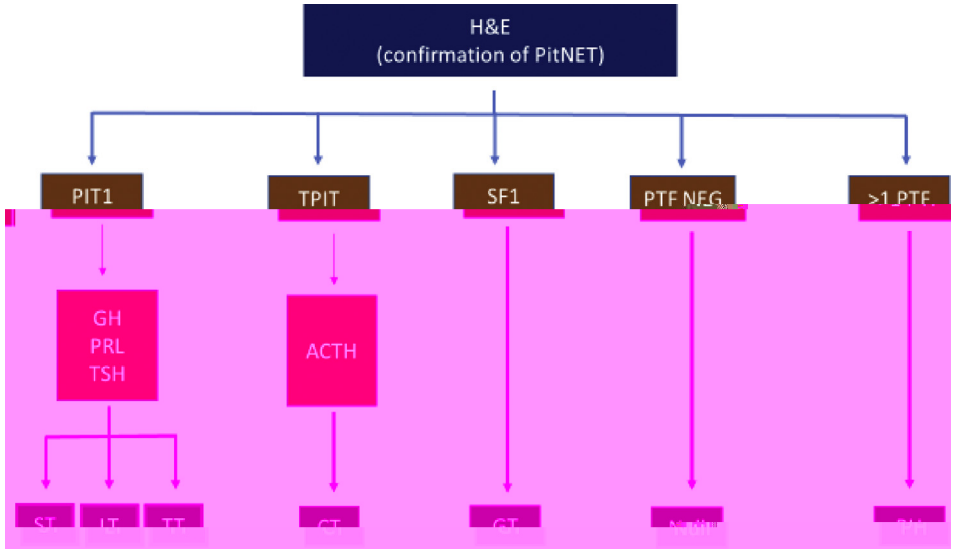


Fig. 4 Diagnostic algorithm based on three pituitary transcription factors. ACTH, Adrenocorticotropin hormonr; PFT, pituitary transcription factor; ST, somatotroph tumor; LT, lactotroph tumor; TT, thyrotroph tumor; CT, corticotroph tumor; GT, gonadotroph tumor; PH, plurihormonal tumor; GH, growth hormone; PRL, prolactin; TSH, thyroid-stimulating hormone; H&E, hematoxylin and eosin; PitNET, pituitary neuroendocrine tumor.

subgroup of null cell cases (or with very low levels of positivity as per IHC) that express PIT1. In their series of 31 cases of silent subtype 3 tumors (3.2% of the total cases of PitNET), Mete et al. [8] describe PIT1 positivity in all of them, while 28 present focal expression of different hormones of acidophil lineage, and 3 were negative for all hormones. This subgroup is characterized by clinically aggressive behavior, and from the ultrastructural perspective, it presents monomorphic cells with sparse granules and nuclear inclusions, described as spheridia; a considerable proportion also has fibrous bodies [9]. For these authors, the silent subtype 3 tumor should therefore be included under the PIT1 lineage [8,9]. In our series, we identified 4 PIT1-positive cases (2.7% of the total) of the 32 tumors that were negative for the six pituitary hormones (19%), including one that was clinically diagnosed as prolactinoma and three that were nonfunctioning. The results reported by other authors range from 1.6% [7] to 6% [11], although some series did not identify any [9]. In general, and similarly to our study, all were macrotumors that were mostly invasive. In any case, PIT1 may be a determining factor in the identification of a subgroup of tumors without expression of pituitary hormones but which potentially behaves more aggressively [1,2,8,9].

The interpretation of the PIT1-SF1 coexpression observed in 3 cases of STs presents several possibilities. First, these tumors could have originated in a stem cell with multilineage differentiation capacity. To date, there have been few well-documented cases of PitNETs with markers of more than one cell line, and these mainly correspond to PIT1 and corticotroph lineage [25–28]. There is only one described case of PIT1-SF1 with positivity for hormones of both lineages and simultaneous presence of a CT, suggesting an origin in stem cells with multiple cytodifferentiation [29]. A second possibility would be to interpret them as double tumors; however, the characteristics of the cases would be more consistent with their inclusion as plurihormonal as per WHO criteria [1]. Another explanation could be related to technical aspects. STs expressing gonadotroph hormones are not unusual in the literature, although in most cases, this positivity is attributed to a cross-reaction between the alpha subunit (often present in DGST) and FSH or LH polyclonal antibodies [1,2,6], which could also be applicable to PTFs. The distribution and proportion between cells positive for PIT1 (>95%) and for SF1 (≥12%), would be indicative of coexpression by the same cell subpopulation and would support its character as a stem cell, in line with suggestions by other authors [29]. CTs make up 15–17% of PitNETs in the largest series [8,18], which is consistent with our results (15.1%). The number of Crooke's cell tumors identified (14% of the CTs) is closer to that reported by Mete et al. [8] (18%) than to the results from the German registry (just 0.2%) [18]. The notably scant information about the expression of TPIT [7,8,30] is related to the low reliability attributed to the available antibodies. In our cohort, the sensitivity of the antibody used to identify TPIT for CTs was 100%. Eleven TPIT-positive tumors were silent (10 being ACTH positive and 1 with null expression of pituitary hormones). Silent CTs constitute 6–43% of all CTs [8,30,31], and more aggressive local behavior has been attributed to them than functioning CT and other nonfunctioning pituitary tumors [7,8,30–32]. In our series, silent CTs were more locally aggressive than functioning ones; however, we could not

assess regrowth or recurrence owing to the cross-sectional design.

Broadly speaking, gonadotroph tumors are the most prevalent type of both PitNETs and nonfunctioning tumors. Mete et al. [8] report SF1 positivity in 96% of gonadotroph tumors. In our series, 50% of the PitNETs were positive for SF1, and three also expressed PIT1. The 70 cases that exclusively expressed SF1 were all characteristically nonfunctioning macrotumors. In addition, 70% were positive for gonadotroph hormones, especially LH (82%). Other authors have reported similar results, with up to 37.6% of SF1-positive tumors negative for gonadotrophins [8]. In addition, SF1 expression enabled us to reclassify 21 (65.6%) null cell tumors as being of gonadotroph lineage. In relation to the variable range of positivity observed with SF1 (24% of the SF1 tumors presented positivity with less than 60% positive cells), the close correlation with the percentage of positive cells for gonadotropic hormones could suggest a relationship with tumor heterogeneity.

Five gonadotroph tumors showed low levels of TSH expression (<30%). All these cases were negative for PIT1, so this result could be related to the anomalous production of reactive TSH in gonadotroph cells or a cross-reaction to the antibody. SF1 thus had a sensitivity and specificity of 100% (excluding three PIT1-positive tumors) for identifying tumors of gonadotroph lineage.

The nonfunctioning PitNETs are a heterogeneous group, with most corresponding to gonadotroph tumors with expression of gonadotroph hormones or of SF1 [7,8,10,12,18,32,33]. A significant number of cases are also PIT1 (1.2−9.3%), TPIT (15−16%), or null cell (1.3−4.6%) tumors [7,8,11,12], a finding with both prognostic and therapeutic implications. In our series, 65.8% of PitNETs were nonfunctioning, and 62.5% of these were gonadotroph tumors. The rest comprised 8 PIT1 tumors (8.3%, including 1 ST, 2 LTs, and 2 TTs), 12 TPITs (12.5%) and 6 (6.3%) null cell tumors. Applying the PTFs enabled the reclassification of 81% of the nonfunctioning tumors classified as null cell by hormonal IHC. These results are in line with what are reported in the literature [8,12].

All the surgical series published have described a prevalence of 10−30% of null cell tumors, as classified by hormonal IHC [18,32,33,34]. The addition of PTFs to the hormonal IHC study of pituitary tumors reduced the prevalence of this type to lower than 5% [1,5,8]. The precise identification of null cell tumors is relevant because they seem to have different biological characteristics than gonadotroph tumors [14]. Of the 32 cases diagnosed as null cell by hormonal IHC, just 6 (4.1%) met the criteria set out in the current classification [1,2,4]. The significant reduction in the number of null cell tumors is therefore one of the main contributions that PTF studies make to the classification of pituitary PitNETs [1,2,6,8,10−12]. In the future, incorporating other transcription factors such as GATA3 could further contribute to reducing the percentage of null cell adenomas [35].

The study with cytokeratins (especially 8/18) is important in the subclassification of SG and DG somatotroph PitNETs [1,4], as commented previously. The TPIT tumors show constant cytokeratin expression, with a diffuse or ring-shaped pattern in Crooke's cell CTs. However, cytokeratin staining in SF1 and null cell PitNETs (and more rarely in the PIT1 group) can be negative (this was the case for 31.7% and 28.2%, respectively, of patients in the series of Mete et al.'[8] and 39% and 83% in ours, applying a cutoff of 5% positivity). Although the general recommendation is to use low-weight cytokeratin [1,4], comparative studies could be limited by the composition of the cytokeratin cocktail applied in each study. Our application of a cocktail limited to cytokeratin 8 and 18 could have led to somewhat lower results than those described in the literature, especially in the referred groups. In any case, IHC negativity for cytokeratins necessitates a differential diagnosis with other pituitary tumors such as posterior pituitary neurocytomas and tumors of extrapituitary origin, such as metastases or olfactory neuroblastoma and sinonasal neuroendocrine tumors [1,6,8,36,37]. Most of these cases could be resolved with the study of PTF expression.

One possible limitation of the study is the application of a cutoff point of 5%, which could determine a lower degree of diagnostic sensitivity in the IHC study. On the other hand, by not including marginal percentages (less than 5%) in the definition of positivity, we avoided defining adenohypophyseal tumor elements included as positive, obtaining greater specificity in the diagnosis of the subtypes. Moreover, the IHC study of PTFs performed on tissue microarrays may also have contributed to reducing the sensitivity of the results, although the percentage obtained from null cases is consistent with that reflected in the literature [8].

We can confirm the utility of SF1 for detecting a substantial portion of gonadotroph tumors, which reduces the estimated prevalence of null cell tumors to less than 5%, and we also identified plurihormonal PitNETs with PIT1-SF1 coexpression, as well as the hormone-negative PIT1s, a group in which we did not observe differences in the clinical behavior compared with the rest of the tumors of the same cell lineage.

In conclusion, our results show that evaluating PTFs allows for a more precise classification of PitNETs, contributing to improvements in treatment and follow-up in these patients, as other groups have also suggested [10]. By adding TPIT assessment with the new antibody, we propose a two-step algorithm (Fig. 4), with hypophyseal hormones being used in a selective modality, depending on initial results. Its validation in larger series will be necessary.

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