Pituitary Neuroendocrine tumor

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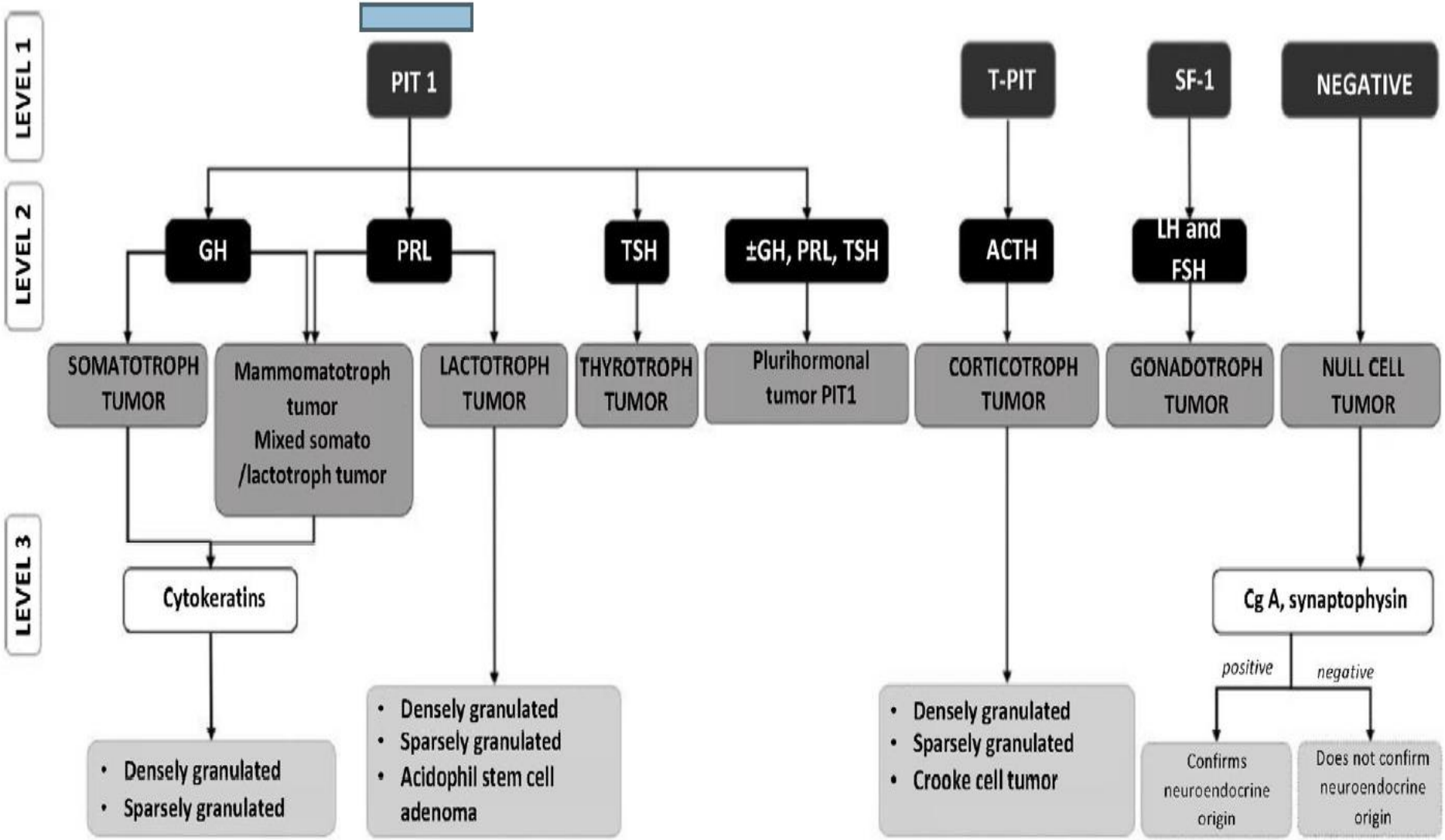
PitNETs

- In the recent 2017 WHO classification groups replacement of the term adenoma by pituitary neuroendocrine tumor (PitNET).
- PitNETs have traditionally been called adenomas because of their usual indolent behavior as compared to other neoplasms and neuroendocrine tumors in other locations.
- According to the authors, this term would better reflect the similarities between adenohypophyseal tumors and neuroendocrine tumors in other organs and the aggressive behavior of some of the former.

Fourth edition of the WHO Classification of Tumors of Endocrine Organs2 was published in 2017

The main changes include:

- It is recommended to measure the PTFs of the three pituitary cell lines: the pituitary-specific transcription factor 1 (Pit-1), the transcription factor T-PIT,and the steroidogenic factor 1 (SF1). Specifically, it has allowed for a drastic reduction in the proportion of null tumors and for a more precise classification of plurihormonal tumors.
- The term atypical adenoma, proposed in the 2004 classification, was removed. In contrast, high risk subtypes, characterized by a greater capacity to invade, relapse, and metastasize than the other PitNETs, are identified.
- The recommendation to determine the MIB1/Ki-67 index as a proliferative marker is maintained, and measurement of p53 is relegated to tumors with Ki-67 ≥ 3%.
- Pituitary blastoma is included as a new entity.



- Tumor types are in uppercase, subtypes in lowercase.
- **The Ki-67 proliferation index (LEVEL 1) should be measured in all cases**. p53 will only be measured when Ki-67 index is ≥3%).
- Measurement of **somatostatin or dopamine receptors or E-cadherin** should be relegated to validation studies and selected cases **(LEVEL 3).** For example, somatotropinomas in which this information may help identify response to drug treatment.
- Plurihormonal tumors may have combinations of adenohypophyseal hormones and transcription factors of different cell lines. They are called unusual plurihormonal tumors. Their clinical significance has not been determined yet.

Non-functioning neuroendocrine pituitary tumors

- PitNETs not associated with a recognizable clinical hormonal syndrome have historically been grouped under the acronym NFPAs.
- Since the proliferation of highly sensitive imaging techniques, non-functioning PitNETs have been the most frequently diagnosed pituitary neuroendocrine tumors in both surgical and non-surgical series.
- NFPAs are most commonly macroadenomas and may cause symptoms related to a mass effect (headache or neurophthalmological changes), partial or total hypopituitarism, and hyperprolactinemia, or be incidentally discovered on a brain MRI.
- When the cell line from which the tumor is derived is identified after surgery and the tumor is not associated to a systemic endocrine syndrome, the tumor is said to be silent.
- Thus, after surgery, the term NFPA should be replaced by the corresponding tumor cell type accompanied by the adjective silent; for example, silent corticotroph PitNET, silent lactotroph, etc., to differentiate it from the respective functional variant.

Silent Pit NET

- The terms silent pituitary adenoma or silent PitNET describe adenohypophyseal tumors that express one or more adenohypophyseal hormones or their transcription factors in immunohistochemistry (IHC) but do not secrete hormones in clinically relevant levels.
- All hormonally active PitNET subtypes have silent homologues that have been grouped under the acronym (non-functioning pituitary adenomas)NFPA.

Silent Pit NET

- Approximately 10% of gonadotroph PitNETs are functioning. The adjectives silent and functioning should therefore also be applied to this subtype of PitNETs.
- Totally silent :circulating levels of the corresponding hormone are normal.
- Clinically silent: elevated circulating hormone levels but without expressing the corresponding clinical syndrome.
- In addition, the functional status of a PitNET may change during follow-up, more frequently in the case of tumors of corticotroph lineage.

High risk adenoma subtypes

The 2017 WHO classification deleted the term atypical adenoma.

Instead, it recognized high-risk adenoma subtypes with a poorer prognosis during follow-up:

- Lactotroph adenomas in males
- Silent corticotroph and Crooke cell adenomas
- Sparsely granulated somatotroph adenomas
- Silent Pit-1 plurihormonal tumors.

Aggressive tumors

However, the concepts of invasion and aggressiveness are often confused, since an invasive tumor may not be aggressive while all aggressive tumors are invasive.

(1) invasion: radiographic or anatomical invasion of the sinuses adjacent to the sella turcica, regardless of Ki-67 expression

(2) proliferation:MIB1/Ki-67 immunostaining ≥3%, regardless of the presence or absence of invasion

(3) aggressiveness: invasive tumor that relapses or grows during follow-up despite adequate treatment, regardless of proliferation markers.

Null cell tumor or immunonegative tumor?

- Null cell tumor does not show specific cell differentiation on IHC of adenohypophyseal hormones or PTFs and molecular gene expression studies of pituitary hormones and their PTFs.
- Null cell tumors consist of chromophobic or somewhat acidophilic cells, arranged in a leaf-shaped pattern. They differ from gonadotropinomas in that papillary or pseudopapillary growth patterns are less common.
- Their current prevalence is less than 3% in all mentioned series.

Null cell tumor or immunonegative tumor?

With the new procedures, most tumors initially considered as null tumors are reclassified as gonadotropinomas (most of them express SF-1) or, less commonly, as silent corticotropinomas.

Since truly null tumors and silent corticotropinomas may behave more aggressively than gonadotropinomas, adequate identification is very important.

Because of immunonegativity for pituitary hormones and PTFs, diagnosis is exclusionary,the current recommendation is to call them immunonegative tumors rather than null tumors

Clinical information that the pathologist should know before the study

Pathological diagnosis is made in a clinical setting, and it is therefore essential that the pathologist receives the necessary background information.

Table 2 Information to be provided by the clinician (endocrinologist/neurosurgeon) to the pathologist together with the tissue sample to be analyzed.

Type of sample	• Intraoperative biopsy • Final tumor tissue • Normal pituitary gland • Other
Suspected diagnosis	• Endocrine clinical syndrome • Non-functioning
Family history	• Multiple endocrine neoplasia, isolated familial pituitary adenoma, Carney syndrome, pheochromocytoma/paraganglioma
Tumor size	• Largest diameter in millimeters
Surgical procedure performed	• Transsphenoidal, microscopic, transcranial
Radiographic characteristics	• Invasion of cavernous sinuses, sphenoidal sinuses, or clivus • T2 intensity
Degree of excision	• Total, partial, biopsy
Prior treatment	• Surgery (repeat) • Radiotherapy (conventional, stereotactic, radiosurgery) • Pharmacological: 1st and 2nd generation somatostatin analogues, dopamine agonists, pegvisomant, ketoconazole

Sample collection and shipping conditions

All tissue obtained should be submitted to the pathology department, and manipulation in the surgical area should be avoided as much as possible.

It is recommended that biological material is sent fresh and

without fixative in a gauze moistened with saline immediately after collection to prevent tissue ischemia.

The specimen should be formalin fixed only after a minute portion, at minimum a single 1-mm fragment, has been placed in glutaraldehyde for possible ultrastructural study.

With an adequate sample, consideration can be given to freezing a portion for biochemical or genetic studies.

When diagnostic tissue is scant or no lesion is identified on cytologic or frozen section assessment, the entire specimen should be step sectioned to obtain H&E and unstained slides.

Gross analysis and processing of tissue

Gross analysis should describe the number of fragments and their approximate volume.

The pathologist is responsible for selecting the tissue to be used for special techniques or for biobanks, always prioritizing the diagnostic needs.

Microscopic control of the sample before freezing is recommended ,and can be done quickly with a cytological sample by crushing a small fragment.

When an intraoperative diagnosis is required, and in order to prevent tissue loss and appearance of artifacts that occur in frozen sections, cytology by smear or crushing of a small fragment is recommended.

Microscopic study

- The tumor tissue for the diagnostic study will be fixed in neutral buffered formalin (for 6---48 h).
- Initial evaluation is performed with hematoxylin-eosin staining to confirm that it is a PitNET.
- The staining type,cell characteristics (degree of pleomorphism), architecture, extent of fibrosis, necrosis, signs of bleeding or any other morphological parameter of interest will be determined.
- Presence of normal pituitary tissue will be reported if it is part of the biological material submitted.
- If there is any doubt that the biological material is from the normal pituitary gland rather than the tumor, a reticulin stain will be performed. This technique is especially recommended for Cushing's disease if no tumor has been identified on MRI.
- The number of mitoses in 10 fields (40×) will be quantified

Normal adenohypophysis

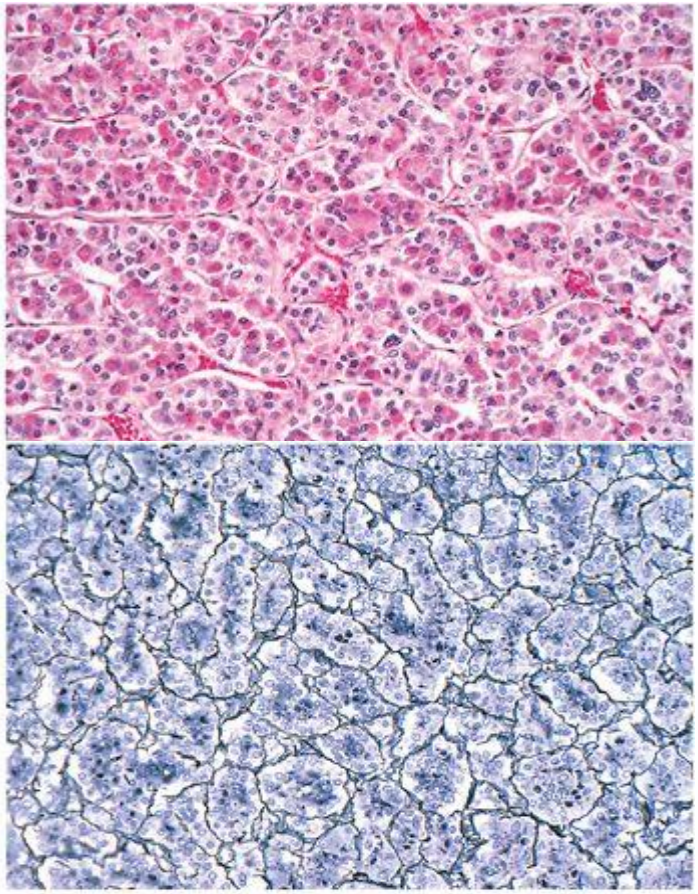


FIGURE 12.3 Normal anterior pituitary. Acini and cords of cells are demonstrated (reticulin stain).

Pituitary adenoma

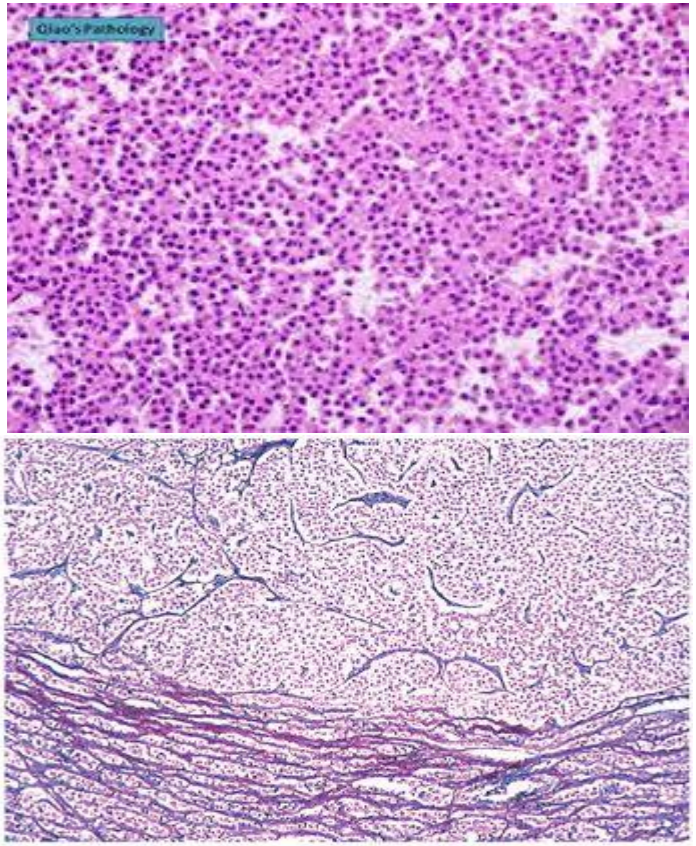


FIGURE 12.14 Pituitary adenoma. The lack of reticulin content and the compression of surrounding parenchyma are demonstrated (reticulin stain).

Immunohistochemical study

- The immunohistochemical study will allow for characterizing the tumor and for determining variables of response to the drug treatment of prognostic interest.
- Positive values in IHC < 5% should be interpreted with caution when a given tumor subtype is classified.
- Luckily, measurement by IHC or molecular techniques of PTFs will allow for adequate typing of these doubtful cases.

Table 3 Multilevel recommendations of the immunohistochemical study of pituitary neuroendocrine tumors according to the recommendations of the European Pituitary Pathology Group[16]

LEVEL 1	PRL/GH/TSH	ACTH	FSH/LH	LMWK[a]	MIB1/Ki-67
Hormones					
Cytokeratins					
Proliferation markers					
LEVEL 2	PIT-1		T-PIT		SF-1
Pituitary transcription factors					
LEVEL 3	CgA[b]		SSTR, E-cadherin		P53
For selected cases only (see main text)					

ACTH: corticotropic hormone; FSH: follicle-stimulating hormone; GH: growth hormone; LH: luteinizing hormone; LMWK: low molecular weight cytokeratins; MIB1/Ki-67: MIB1 proliferation index; PIT1: pituitary-specific transcription factor 1; PRL: prolactin; SF-1: steroidogenic factor 1; SSTR: somatostatin receptors; TPIT: T-box transcription factor; TSH: thyroid-stimulating hormone.

[a] Study indicated for somatotroph and corticotroph tumors.

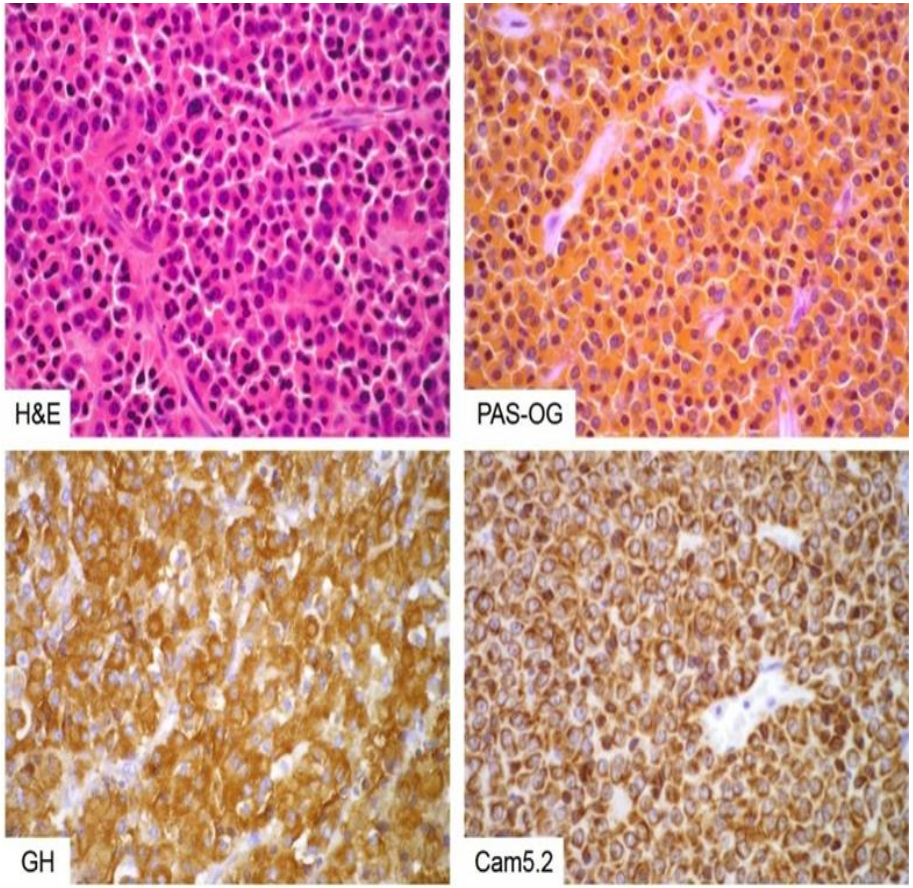
[b] Chromogranin (CgA) measurement is mandatory for all immunonegative or poorly positive tumors.

Quantification by cytokeratin immunohistochemistry

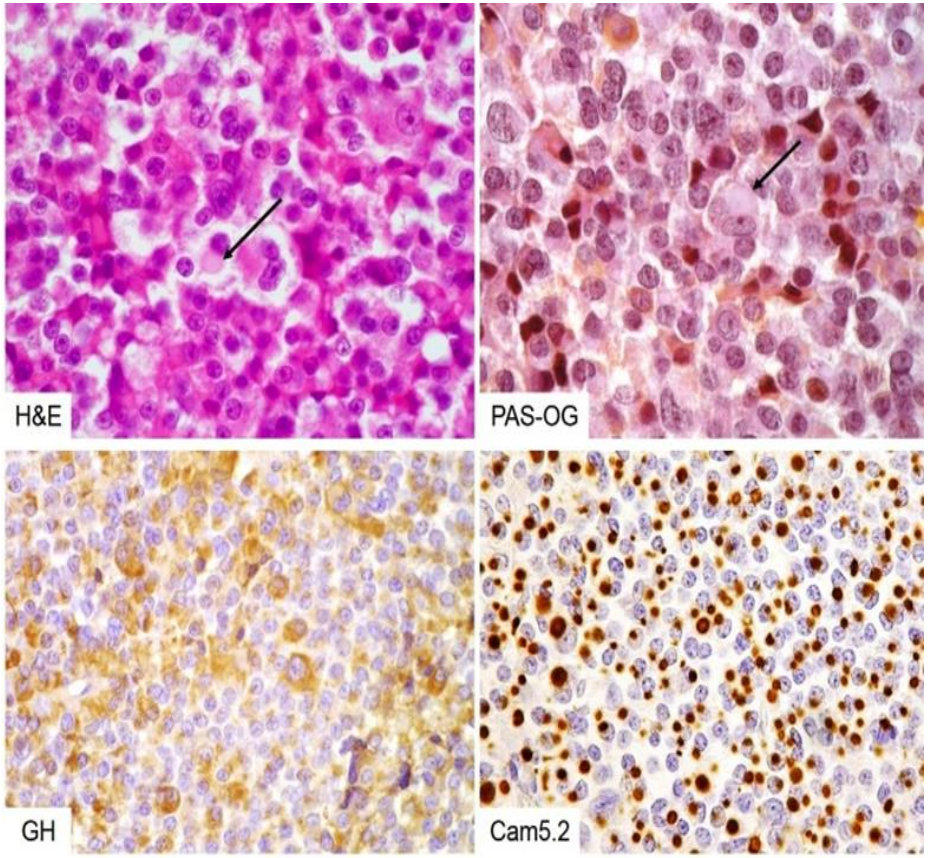
- Measurement of expression of low molecular weight cytokeratins, particularly cytokeratins 8 and 18, usually identified using the antibody CAM5.2 (CK8 and CK7), is useful for consistent identification of some variants of the PitNET subtypes, particularly the two main somatotropinoma subtypes.
- A diffuse perinuclear pattern of cytokeratins is diagnostic of densely granular somatotropinoma, while a dot-like pattern with a proportion of fibrous bodies >70% identifies sparsely granular somatotropinomas.
- Differentiation and inclusion of these two patterns in the pathology report is relevant for clinicians, because densely granular somatotropinomas have a better response to first-generation somatostatin analogues.
- An abundant cytoplasmic expression pattern is also associated with corticotroph PitNETs.
- However, most adenomas negative for cytokeratins are gonadotroph adenomas, and cytokeratin measurement would not be indicated in this subtype.

IHC of somatotroph adenoma

Densely granulated



Sparsely granulated

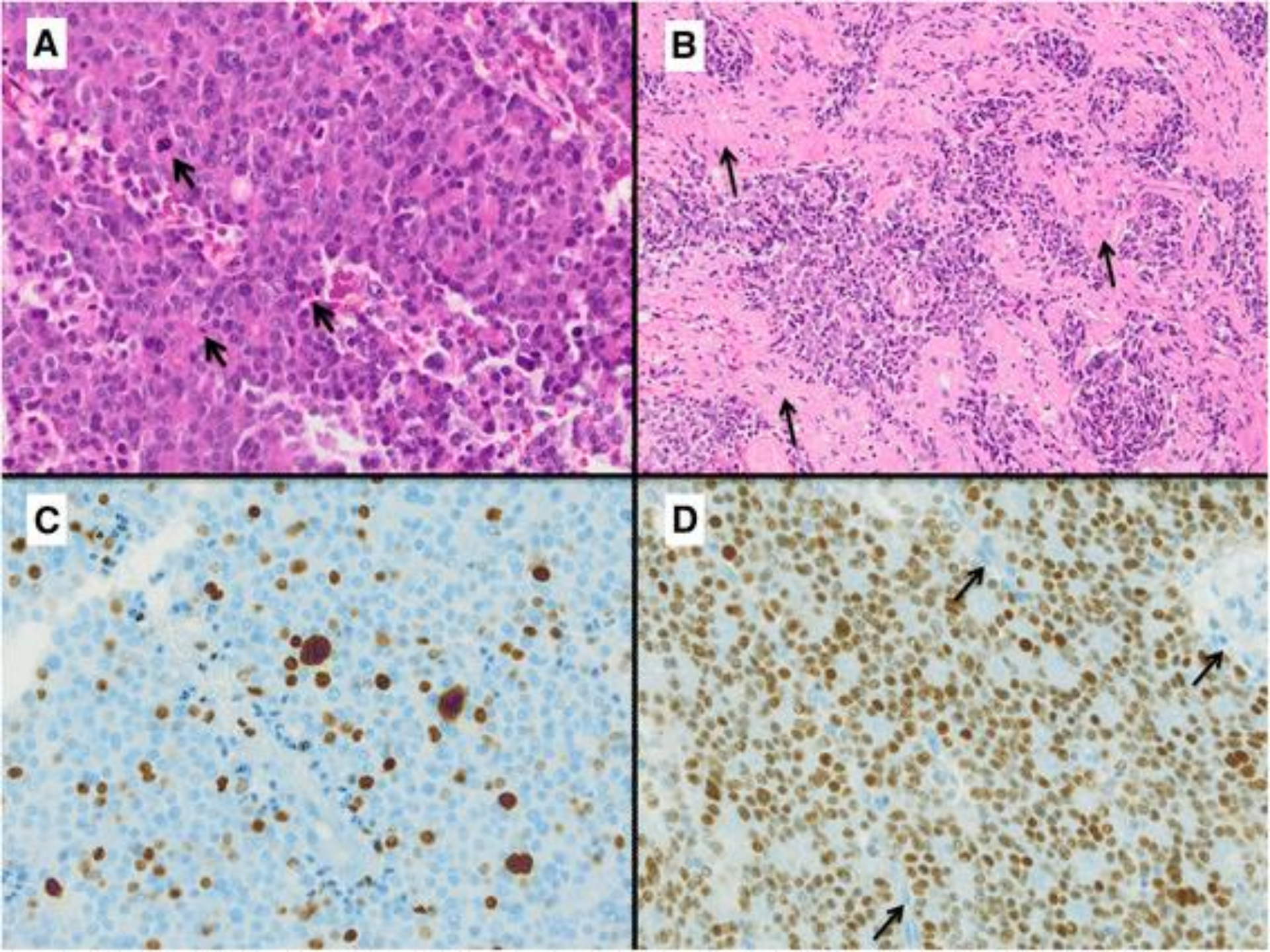


Classification of pituitary neuroendocrine tumors.

Type of adenoma or PitNET	Morphological variant	Pituitary hormones and other immunomarkers	PTFs and other cofactors
Somatotroph adenoma	Densely granulated	GH ± PRL ± subunit α	PIT-1
		Perinuclear or diffuse cytokeratin pattern	
	Sparsely granulated	GH ± PRL, dot-like pattern of cytokeratins	PIT-1
	Mammosomatotroph	GH + PRL (in same cells) ± subunit α	PIT-1, ERα
	Mixed somatotroph-lactotroph	GH + PRL (in different cells) ± subunit α	PIT-1, ERα
Lactotroph adenoma	Densely granulated	Diffuse PRL	PIT-1, ERα
	Sparsely granulated	Perinuclear PRL	PIT-1, ERα
	Acidophilic stem cell	PRL, GH (focal and variable)	PIT-1, ERα
		Dot-like pattern of cytokeratins	
Thyrotroph adenoma		β-TSH, subunit α	PIT1, GATA2
Corticotroph adenoma	Densely granulated[a]	ACTH	T-PIT
		Diffuse pattern of cytokeratins	
	Sparsely granulated	ACTH	T-PIT
		Diffuse pattern of cytokeratins	
	Crooke cell	ACTH	T-PIT
Gonadotroph adenoma		β-FSH, β-LH ± subunit α	SF-1, CAT2, ERα
Null cell adenomas		None	None
Plurihormonal adenoma	PIT-1 plurihormonal adenoma	GH, PRL, β-TSH ± subunit α	PIT-1
	Adenomas with unusual combinations	Miscellaneous combinations: ACTH/GH, ACTH/PRL	N/A

Assessment of proliferation

- MIB1-LI/Ki-67 index It is important that cell proliferation is estimated by immunohistochemical quantification of the MIB1-LI/Ki-67staining index because this has been considered as a possible prognostic factor in PitNETs .
- Quantification with Ki-67 will be performed in two of the areas of the preparation with the greatest number of hot spots, considering between 500 and 1000 cells,and will be expressed as a percentage over the total number of tumor cells.
- Unlike for other neuroendocrine tumors, no cut-off point that accurately predicts aggressive behavior of the tumor has been established in PitNETs.
- It is accepted that most aggressive tumors have a Ki-67 ≥ 3% , and if Ki-67 is greater than 10%, the possibility of sellar metastasis or a pituitary carcinoma should be considered.


A
B
C
D

- Quantification of the number of mitoses in PitNETs is not as important as for grading neuroendocrine tumors of the GI tract.
- The result is expressed per square millimeter, which is equivalent to 5 high-power fields (40×),and presence of two mitoses per 10 high-power fields is relevant.
- p53 positivity in tumor tissue suggests mutations or inactivation of the suppressor gene TP53 and is considered a marker of aggressive behavior in many solid tumors. Its prognostic significance in PitNETs is however limited. Measurement of p53 is therefore only recommended in tumors with a Ki-67 index ≥3%

Table 5 Clinical-pathological classification of pituitary neuroendocrine tumors according to Trouillas et al., 2013.[9,14]

The classification is based on 3 characteristics:

1. Tumor diameter as micro (<10 mm), macro (≥10 mm) or giant (≥40 mm) according to MRI study

2. Type of tumor according to immunohistochemical study: GH, PRL, ACTH, FSH/LH, and TSH

3. Tumor grade based on the following criteria:

Invasion: defined as histological or radiographic (MRI) evidence of cavernous sinus or sphenoid invasion

Proliferation: based on presence of at least 2 of the 3 criteria:

- *Ki-67*: >1% (Bouin-Hollande fixative) or ≥3% (formalin fixative)
- *Mitoses*: *n* >2/10 high-power fields
- *P53*: positive (>10 strongly positive nuclei/10 high-power fields)

Grades of tumor behavior established according to previous characteristics:

Grade 1a	Non-invasive tumor
Grade 1b	Non-invasive and proliferative tumor
Grade 2a	Invasive tumor
Grade 2b	Invasive and proliferative tumor
Grade 3	Metastatic tumor (cerebrovascular or systemic metastases)

High-power field: 0.30 mm^2, 400× magnification.

ACTH: corticotropic hormone; FSH: follicle-stimulating hormone; GH: growth hormone; LH: luteinizing hormone; PRL: prolactin; MRI: magnetic resonance imaging; TSH: thyroid-stimulating hormone.

Other immunohistochemical measurements

In null tumors, immunostaining of chromogranin A (CgA) and synaptophysin may be helpful to certify the neuroendocrine origin of the lesion and differentiate it from other tumors in the sellar region.

While several publications suggest that expression of the pituitary tumor transforming gene (PTTG) would help predict aggressiveness of PitNETs, this has not been supported by large series. Measurement of PTTG is therefore not advised.

somatostatin receptor و E-cadherin

- A positive correlation has been observed between SSTR2 , densely granulated adenomas and reduction in GH after SSA treatment .
- while SSTR5 was associated with sparsely granulated tumors

- E-cadherin (lost in sparsely granulated) allows for more accurate identification of the response to somatostatin analogues than SSTR expression.
- However, treatment algorithms for acromegaly do not include yet routine IHC measurement of SSTR or E-cadherin in daily clinical practice.
- These substances are therefore only measured for the time being for research purposes or in duly justified specific cases.

Methylguanine methyltransferase

(MGMT), the DNA repair enzyme that attenuates the effect of in the treatment of aggressive carcinomas and PitNETs, has been proposed as a marker of negative response to temozolomide.

Recent data have not confirmed that MGMT expression, IHC, methylation of the MGMT promoter or proliferative index values with Ki-67 or p53 are predictors of response to temozolomide.

No agreement on how to assess MGMT.

Routine measurement of MGMT is therefore not currently warranted as a predictor of response.

Loss of MSH6

Alternatives proposes to MGMT testing include evaluation of DNA repair proteins, particularly MSH6, whose expression has been associated with response to temozolomide.

Loss of nuclear immunoexpression of MSH6, and also of MSH2, has been associated with loss of efficacy of treatment with temozolomide.

Raf kinase inhibitor protein(RKIP)

- low RKIP expression levels and lack of RAF kinase inhibition are associated with a poorer response to somatostatin analogues in somatotropinomas.

In recent years, other cell proliferation, apoptosis, and angiogenic markers, oncogenes, tumor suppressor genes, cell cycle mediators, long non-coding microRNAs and RNAs have been identified that participate in tumorigenesis, migration, proliferation, and invasive capacity of pituitary adenomas have been identified.

Future contributions of molecular biology

However, no reliable morphological markers that predict PitNET recurrence are yet available. Recent research has provided new insights into the genesis and biological behavior of these tumors.

The molecules explored include the TACSTD family (EpCAM, TROP2), neuropiline (NRP-1), oncogene-induced senescence (OIS), faskines (FSCN1), genes associated with invasion (CLDN7, CNTNAP2, ITGA6,JAM3, PTPRC, and CTNNA1) EZH2, and ENC1 and endocangenes.

Molecular techniques, bioinformatics, and new drug options are useful tools for expanding understanding of the complex nature of pituitary tumorigenesis.

As their clinical value is established, they will have to be included in the pathology report.

Table 4 Proposed pathology analysis report for PitNETs.

Clinical and preoperative information				
Functioning adenoma ☐ Yes / ☐ No	**Preoperative treatment** ☐ Surgery ☐ Dopamine agonists ☐ Somatostatin analogues	☐ Pegvisomant ☐ Ketoconazole ☐ Antithyroid drugs	☐ Radiotherapy	**Relapse** ☐ Yes / ☐ No
Surgical procedure performed				
Preoperative MRI available? ☐ Yes / ☐ No		**Largest diameter in millimeters**		
Radiological invasion:	**Cavernous sinus** ☐ Yes ☐ No ☐ Unknown	**Sphenoidal sinus** ☐ Yes ☐ No ☐ Unknown	**Other bone** ☐ Yes ☐ No ☐ Unknown	
Macroscopic examination				
Size of specimen				
No. of fragments fixed in_____*(state fixative)*				
No. of fragments cryopreserved				
Histological examination				
Hematoxylin-eosin	**Cell type** ☐ Acidophilic ☐ Basophilic ☐ Chromophobic	**Pattern** ☐ Diffuse ☐ Trabecular ☐ Pseudopapillary ☐ Other_____ ☐ Adenohypophysis ☐ Neurohypophysis	**Other characteristics** ☐ Oncocytic change ☐ Calcification ☐ Inflammation ☐ Other: ☐ Necrosis ☐ Fibrosis	
Reticulin stain Contains non-tumoral pituitary gland: ☐ Yes / ☐ No			**Crooke cells** ☐ In adenohypophysis	☐ Yes / ☐ No ☐ In adenoma
Mitotic cell count (no. per square millimeter. 40X magnification)				
Histological infiltration (if applicable)			**Dura** **Bone** **Respiratory mucosa**	☐ Yes / ☐ No ☐ Yes / ☐ No ☐ Yes / ☐ No
Immunohistochemistry (antibody panel, note clone reference)				
Hormones	GH_____% PRL_____%	TSH_____% ACTH_____%		LH_____% FSH_____%
Pattern of cytokeratins	☐ Diffuse cytoplasmic ☐ Dot-like pattern ☐ Membrane	☐ Perinuclear	☐ Negative	
Proliferation markers	**MIB-1/Ki-67 Index** % positive cells			
Pituitary transcription factors (include percentage if positive)	**PIT-1** ☐ positive_____% ☐ negative	**T-PIT** ☐ positive_____% ☐ negative		**SF-1** ☐ positive_____% ☐ negative
Chromogranin A (CgA)		☐ positive / ☐ negative		
p53 (if MIB1/Ki-67 index ≥3%)*			%	
Other techniques				
Final diagnosis (WHO 2017)				
Morphological classification with/without evidence of proliferation (mitotic count and Ki-67 index)[2]				
Integrated diagnosis with grades 1a, 1b, 2a, 2b, 3 (Table 5) [13,14]				

*Optional and conditional on Ki-67 proliferation index.