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REVIEW AND PERSPECTIVES

# A standardised diagnostic approach to pituitary neuroendocrine tumours (PitNETs): a European Pituitary Pathology Group (EPPG) proposal

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## Abstract

The 2017 World Health Organization (WHO) classification proposes to type and subtype primary adenohypophyseal tumours according to their cell lineages with the aim to establish more uniform tumour groups. The definition of atypical adenoma was removed in favour of high-risk adenoma, and the assessment of proliferative activity and invasion was recommended to diagnose aggressive tumours. Recently, the International Pituitary Pathology Club proposed to replace adenoma with the term of pituitary neuroendocrine tumour (PitNET) to better reflect the similarities between adenohypophyseal and neuroendocrine tumours of other organs. The European Pituitary Pathology Group (EPPG) endorses this terminology and develops practical recommendations for standardised reports of PitNETs that are addressed to histo- and neuropathologists. This brief report presents the results of EPPG's consensus for the reporting of PitNETs and proposes a diagnostic algorithm.

**Keywords** Standardised diagnostic approach · Pituitary neuroendocrine tumours · European Pituitary Pathology Group

The 2017 World Health Organization (WHO) classification based the typing and subtyping of sellar neuroendocrine and non-neuroendocrine tumours on pathological criteria and suggested the use of lineage-restricted pituitary transcription factors (TFs) for diagnostic practice. New entities were introduced, the term "atypical adenoma" removed and some tumour types and subtypes are now regarded as intrinsically aggressive [1].

A group of European pathologists, endocrinologists and scientists with interest and expertise in sellar pathology established in 2016 the European Pituitary Pathology Group (EPPG). The EPPG has recently been endorsed by the European Neuroendocrine Association (https://www.eneassoc.org). The aims of EPPG are to (i) provide collegial review of challenging cases; (ii) propose standardised reports and diagnostic algorithms to improve diagnostic practice; (iii) standardise immunohistochemistry (IHC) protocols; (iv) refine prognostic and predictive markers for sellar tumours; and (v) promote the discipline among young pathologists.

In this brief report, we propose a standardised format (Fig. 1) and diagnostic algorithm (Fig. 2) as practical tools to diagnose pituitary neuroendocrine tumours (PitNETs) in both children and adults. Future EPPG activities will concentrate on rare PitNETs, IHC standardisation, markers of aggressiveness and treatment response and on standardised reports for sellar non-neuroendocrine tumours.

## From adenoma to pituitary neuroendocrine tumour

The EPPG endorses the consensus reached at the International Pituitary Pathology Club in 2016 to replace the term adenoma with PitNET [2] and to align PitNETs with the other neuroendocrine neoplasm (NEN), as recently suggested by the WHO panel of NEN experts [3].

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## EPPG PROPOSAL FOR STANDARDISED HISTOLOGICAL REPORT FOR PitNETs

**1. Pre-operative information**

1.1 Endocrine status: ..... Pre-operative treatment: ..... Recurrent tumour : (yes/no)

1.2 Preoperative MRI – Available (yes/no) Maximum dimension: mm .... Normal.... Micro.... Macro.... Giant....

Invasion : cavernous sinus (yes/no/unknown), sphenoidal sinus (yes/no/unknown), other bone (yes/no/unknown),

**2. Macroscopy**

2.1 Number of fragments fixed in ..... (note the fixative) :

2.2 Number of fragments cryopreserved : .....

**3. Histology**

3.1 HE stain: Adenohypophysis: (yes/no) Neurohypophysis: (yes/no) Other features: oncocytic changes/calcification/inflammation/necrosis/cyst/fibrosis

3.2 For Cushing's disease : Reticulin stain or Collagen IV if the tumour is not evident Crooke's cells: (yes/no)

3.3 Mitotic count : number of mitoses per 10 high power field (40X)

3.4 Histological invasion: dura-matter (yes/no) ; bone (yes/no); respiratory mucosa (yes/no)

**4. Immunohistochemistry**

Panel of antibodies (note the reference of the clones) :

•Hormones: PRL ...%, GH ...%, TSH ...%, ACTH ...%, FSH ...%, LH ...%,

•Cytokeratin LMWK pattern: fibrous bodies (>70% yes /no); cytoplasmic (yes/no); negative

•Proliferation markers: MIB1/Ki67 index (counted in hot spots): ...%

•Transcription factors : PIT1 positive/negative, TPIT positive/negative, SF1 positive/negative

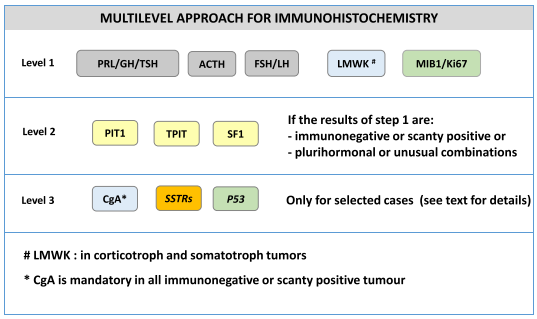
•Chromogranin A (CgA) : positive/negative

•If required :

•P53 (if MIB1/Ki67≥3%): negative / ...%

•SSTRs (score) : .....

•ERα (score) : .....




**5. Diagnosis of PitNET**

**Morphological classification with/without signs of proliferation** (mitotic count and Ki-67 index) (Ref. 1)

**Integrated diagnosis with grades 1a;1b;2a;2b,3°** (Ref. 13-14)

° When indicated MGMT status assessed: see text

**Fig. 1** EPPG proposal for standardised histological report for PitNETs

## Proposed standardised histological reports

For safe and accurate diagnosis of PitNETs, the EPPG recommends a multi-step approach (Fig. 1), comprising the summary of clinical and neuroimaging features, IHC for hormones and TFs, assessment of proliferation and, when indicated, the use of markers predictive of treatment response. We emphasise that assessment of sellar pathology requires adequate training and an integrated, multidisciplinary approach that involves endocrinologists, neuroradiologists, neurosurgeons, geneticists and oncologists.

### Step 1: Clinical and neuroimaging features should be integrated in the pathology report

The EPPG recommends endocrine status, pre-operative pharmacological treatment and recurrence to be summarised in the pathology report (Fig. 1—1.1). Family history and genetic syndromes should be checked, especially in patients younger than 30 years [4, 5]. Tumour size and invasion of surrounding structures should also be recorded (Fig. 1—1.2). Although we acknowledge that it is not in the remit of pathologists to interpret clinical and neuroimaging features, this information is relevant to a conclusive diagnosis in cases of clinically silent tumours and to identify aggressive PitNETs as defined by the European Society of Endocrinology guidelines [6, 7].

### Step 2: Definition of type and subtype

Haematoxylin-eosin (H&E)-stained sections provide an essential overview of architecture, cellularity and cytological features such as oncocytic change, necrosis and inflammation. The presence of normal anterior and posterior pituitary gland should be recorded (Fig. 1—3.1). In patients with Cushing's disease (CD), reticulin staining or collagen IV IHC is suggested for delineation of PitNET and residual anterior pituitary

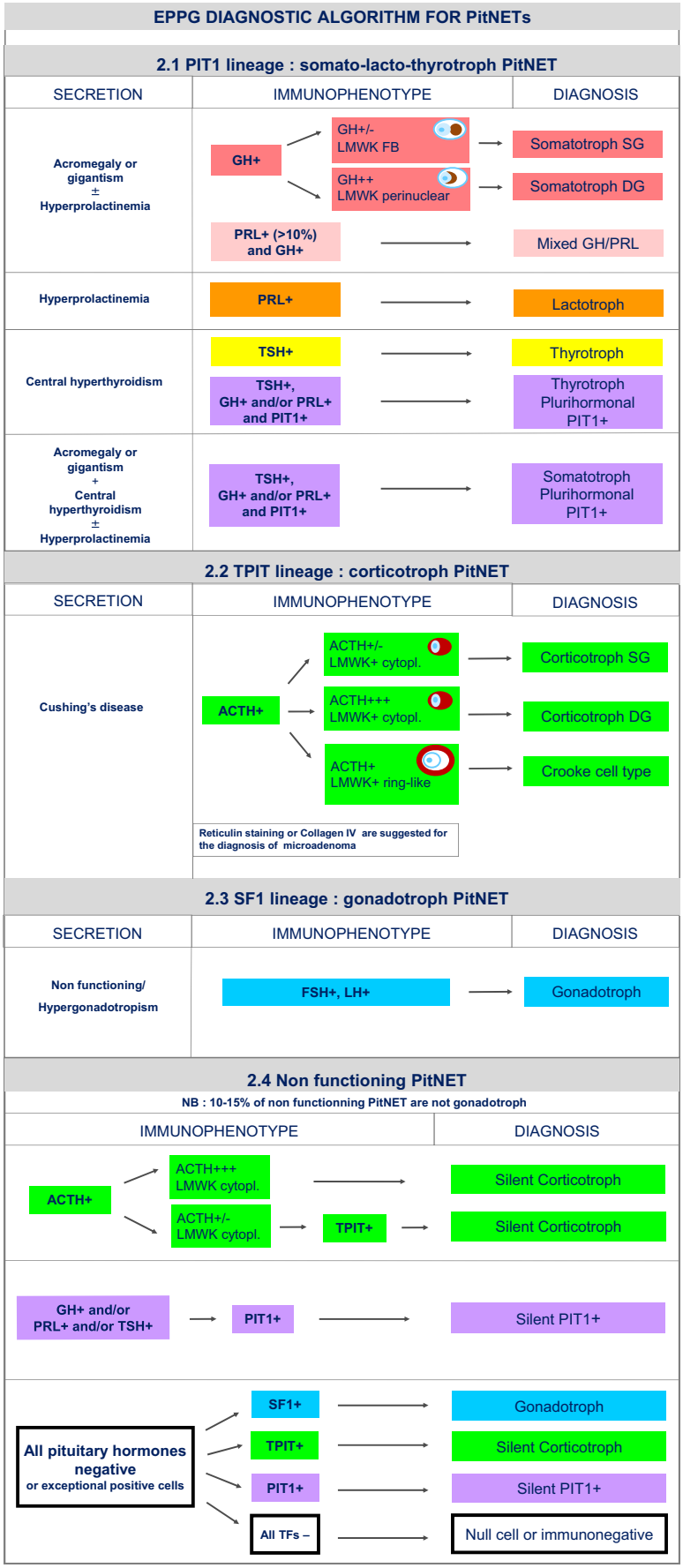



**Fig. 2** EPPG diagnostic algorithm for PitNETs. Used abbreviations: GH growth hormone, LMWK low molecular weight cytokeratin, FB fibrous bodies, SG sparsely granulated, DG densely granulated, PRL prolactin, TSH thyroid stimulating hormone, PIT1 pituitary-specific positive transcription factor 1, ACTH adrenocorticotroph hormone, FSH follicle stimulating hormone, LH luteinising hormone, TPIT T-box transcription factor, TF transcription factor, SF1 steroidogenic factor-1

gland (Figs. 1—3.2 and 2—2.2). In line with the current WHO classification, we recommend the full panel of IHC for pituitary hormones (Fig. 1—4). PitNET types and subtypes should be defined by the pattern of pituitary hormones and expressed as approximate percentages of positive cells (Fig. 1—4 and Fig. 2). The hormonal profile remains crucial to clinical management.

IHC for low molecular weight cytokeratin (LMWK) should be used to refine the diagnosis of certain PitNET types and subtypes. Intense cytoplasmic LMWK expression is consistent with corticotroph PitNET (silent or CD), perinuclear pattern suggests densely granulated somatotroph and abundant (> 70%) "fibrous bodies" are characteristic of the sparsely granulated variant of somatotroph PitNETs (Fig. 1—4 and Fig. 2—2.1, 2.2, 2.4). Gonadotroph PitNETs usually show limited or even absent LMWK expression. Crooke's hyaline changes in normal corticotrophs should also be reported as they confirm hypercortisolism [8].

TFs are useful but still require diagnostic validation. Assessment based primarily or exclusively on TFs may be misleading. The algorithm proposed by McDonald et al. [9] should be retested with newly available anti-TPIT and PIT1 antibodies and integrated with MIB1/Ki67 assessment.

Microscopic invasion of the dura mater, bone or respiratory mucosa are not validated markers of aggressiveness but are useful to map tumour invasion (Fig. 1—3.4) and correlation with pre-operative MRI and intraoperative observations by the surgeon.

Electron microscopy is unlikely to add any relevant information to the diagnosis of type or subtype. When required, samples can be reprocessed from paraffin.

### Step 3: Assessment of mitotic activity and proliferation

Similar to other NENs, mitotic count and proliferation using MIB1/Ki67 IHC must be carefully assessed. Mitoses should be counted per $mm^2$ (accounting for differences in microscopes, five fields at × 40 equate to about 1 $mm^2$) (Fig. 1—3.3). The MIB1/Ki67 labelling index should be measured in two hotspots [1], and all labelled neoplastic cells should be counted regardless of intensity. Lymphocytes, endothelial cells and stromal cells must be avoided (Fig. 1—4). The number of positive nuclei should be expressed as the percentage (index) of the overall number of neoplastic cells. We recommend counting between 500 and 1000 tumour cells per hotspot [10]. A cut-off of ≥ 3% is currently considered to bear prognostic relevance. A MIB1/Ki67 exceeding 10% requires further investigations to exclude a metastatic deposit or rare non-endocrine primary sellar neoplasms of melanocytic or mesenchymal lineage (Fig. 1—4).

### Step 4: Assessment of tumours that are negative for pituitary hormones and the diagnosis of "null cell" PitNET

In the EPPG members' experience, about 15–20% of PitNETs can be challenging as they show limited or absent expression (Fig. 2—2.4) or unusual combinations of pituitary hormones (e.g. ACTH-GH). In hormone-negative cases, the neuroendocrine origin should be confirmed with chromogranin A and the three TFs IHC (Fig. 1—4 and Fig. 2—2.1, 2.4). In particular, TFs help diagnose a subset of gonadotroph and corticotroph PitNETs and plurihormonal PIT1-positive PitNET (Fig. 2—2.4).

Plurihormonal PIT1-positive PitNETs encompass clinically silent and functioning plurihormonal tumours with acromegaly and central hyperthyroidism and/or hyperprolactinaemia. A potential pitfall of this diagnosis is that the clinical presentation and evolution of these tumours may differ. We suggest that potential differences in the behaviour of silent vs functioning plurihormonal PIT1-positive PitNETs should be further studied.

The 2017 WHO classification revised the concept of "null cell adenoma" and defined it as a tumour without immunohistochemical evidence of pituitary hormone and TFs expression. With this definition, the reported incidence of "null cell" PitNETs dropped to about 1% [11] as many tumours previously designated "null cell" express SF1 and can be assigned to the gonadotroph lineage. We underline the importance of ruling out other primary or metastatic sellar tumours before diagnosis of "null cell" PitNET is made. It is worth remembering that normal adenohypophyseal cells can be entrapped in non-neuroendocrine tumours mimicking plurihormonal PitNETs [12].

### Step 5: Definition of aggressive PitNET

The 2017 WHO no longer recommends the term "atypical adenoma" to define PitNETs with potentially adverse biological behaviour. Concurrent with the European Society of Endocrinology's recent guidelines [6], the EPPG proposes an integrated clinico-pathological approach for aggressive PitNETs that includes invasion as defined by pre-operative MRI/endoscopic intraoperative examination along with MIB1/Ki67 index ≥ 3% and mitotic count > 2/10HPF [13]. Expression of p53 has not been validated as an independent marker of aggressive behaviour but is frequently associated to high Ki67 index. Trouillas and colleagues' approach [13] has been validated in separated studies and can therefore be introduced in diagnostic practice (Fig. 1—5) [6, 7, 14, 15]. Type and subtype as defined by the WHO may not have prognostic implications; for instance, the potential aggressiveness of silent corticotroph PitNETs has not been proven [16].

### Step 6: Markers that guide treatment

Somatostatin receptor (SSTs) status may predict the response to treatment with first- and second-generation somatostatin analogues (SSA). However, there is debate among endocrinologists about the potential benefits of systematic evaluation of SSTs on tissue. Therefore, the EPPG suggests making $SST_2$ and $SST_5$ IHC available when requested. The prognostic value of low oestrogen receptor alpha expression (score < 6) in lactotroph tumours needs to be confirmed [17].

Temozolomide (TMZ) is currently used in patients with aggressive PitNETs. Based on the experience in gliomas, low MGMT protein expression is expected to correlate with a better response to the drug. However, such correlation is not robust in PitNETs; the predictive values of MGMT status in the treatment of aggressive PitNETs and how MGMT should be assessed are still debated [6].

## Proposed diagnostic algorithms

The diagnostic algorithms proposed in Fig. 2 are designed to guide pathologists to a safe and accurate diagnosis. Rare subtypes such as mammosomatotroph and acidophil stem cell PitNETs are not included. Subtypes are clustered following the three main pituitary lineages PIT1, TPIT and SF1 (Fig. 2). Figure 2—2.4 shows an algorithm designed for clinically non-functioning PitNETs, excluding gonadotroph tumours.

## Other recommendations

In line with the guidelines for Pituitary Centres of Excellence [18], we recommend cryopreservation of tissue fragments to facilitate further investigations and support research.

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**Compliance with ethical standards** This manuscript aims at proposing a set of recommendation for safe reporting of pituitary neuroendocrine tumours. It does not involve any use of human or animal tissue. The manuscript has been written in accordance with the ethical framework of each University and in line with the regulations on authorships and plagiarism enacted in each Institution.

**Conflict of interest** The authors declare that they have no conflict of interest.

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