Protocol for the Examination of Specimens from Patients with Pituitary Neuroendocrine Tumor (PitNET)

Version: 1.0.0.0
Protocol Posting Date: March 2025
The use of this protocol is recommended for clinical care purposes but is not required for accreditation purposes.

This protocol should be used for the following procedures AND tumor types:

Procedure	Description
Resection	Pituitary tumor resection (eutopic, ectopic / invasive or arising in a teratoma)
Tumor Type	**Description**
Pituitary neuroendocrine tumor (PitNET)	Corticotroph tumors, Somatotroph tumors, Lactotroph tumors, Mammosomatotroph tumor, Thyrotroph tumor, Immature PIT1-lineage tumor, Mature plurihormonal PIT1 lineage tumor, Mixed somatotroph and lactotroph tumor, Acidophil stem cell tumor, Gonadotroph tumor, Null cell tumor, Multilineage pituitary tumors (Plurihormonal pituitary neuroendocrine tumors with no distinct cell lineages), pituitary neuroendocrine tumor, NOS

The following tumor types should NOT be reported using this protocol:

Tumor Type
Pituicytoma family tumors
Craniopharyngiomas
Rathke's cleft cyst

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<u>**Glossary:**</u>
Author: Expert who is a current member of the Cancer Committee, or an expert designated by the chair of the Cancer Committee.
Expert Contributors: Includes members of other CAP committees or external subject matter experts who contribute to the current version of the protocol.

Accreditation Requirements
The use of this case summary is recommended for clinical care purposes but is not required for accreditation purposes. The core and conditional data elements are routinely reported. Non-core data elements are indicated with a plus sign (+) to allow for reporting information that may be of clinical value.

Summary of Changes

v 1.0.0.0

- New protocol

Reporting Template
Protocol Posting Date: March 2025
Select a single response unless otherwise indicated.
CASE SUMMARY: (Pituitary Neuroendocrine Tumor)

CLINICAL (Note A)

+Clinical History (specify): _____

+Functional Status
___ Functional (specify hormone excess and / or endocrine syndrome): _____
___ Non-functional
___ Other (specify): _____
___ Not specified

+Tumor Size from Imaging Studies
___ Greatest dimension of tumor size from imaging studies in Centimeters (cm): _____ cm
   +Additional Dimension of Tumor Size from Imaging Studies in Centimeters (cm): ____ x ____ cm
___ Other (specify): _____
___ Not specified

+Tumor Extent or Invasive Growth from Imaging Studies
___ Specify extent or invasive growth: _____
___ Not specified

SPECIMEN (Note B)

Procedure
___ Transsphenoidal pituitary tumor resection specimen
___ Transcranial pituitary tumor resection specimen
___ Other (specify): _____
___ Not specified

+Specimen Received
___ Fresh
___ Formalin fixed
___ Other (specify): _____

+Specimen Integrity
___ Intact
___ Fragmented
___ Other (specify): _____

+Specimen Size
___ Greatest dimension in Centimeters (cm): _____ cm
   +Additional Dimension in Centimeters (cm): ____ x ____ cm

___ Other (specify): _____
___ Not specified

TUMOR

Tumor Number (Note C)
___ Single pituitary neuroendocrine tumor
___ Multiple pituitary neuroendocrine tumors
___ Other (specify): _____

Tumor Characteristics

*For multiple pituitary neuroendocrine tumors, repeat the following 5 elements for each distinct pituitary
neuroendocrine tumor (Tumor Types and Subtypes, Tumor Proliferative Activity, Histologically Confirmed
Invasion, Histochemical Features, and Immunohistochemical Features). This section can be repeated up to 10
times.*

Histologic Tumor Type(s) and Subtype(s) (Note D)
___ TPIT-lineage pituitary neuroendocrine tumor (corticotroph tumor)
  ___ Densely granulated corticotroph tumor
  ___ Sparsely granulated corticotroph tumor
  ___ Crooke cell tumor
  ___ Other (specify): _____
___ PIT1-lineage pituitary neuroendocrine tumor
  ___ Somatotroph tumor, densely granulated
  ___ Somatotroph tumor, sparsely granulated
  ___ Lactotroph tumor, densely granulated
  ___ Lactotroph tumor, sparsely granulated
  ___ Thyrotroph tumor
  ___ Mammosomatotroph tumor
  ___ Mature PIT1-lineage plurihormonal tumor
  ___ Immature PIT1-lineage tumor
  ___ Acidophil stem cell tumor
  *# For each tumor component specify tumor subtype as listed above for somatotroph and lactotroph tumors
  (e.g., mixed sparsely granulated somatotroph tumor and sparsely granulated lactotroph tumor).*
  ___ Mixed somatotroph and lactotroph tumor# (specify subtype): _____
  ___ Other (specify): _____
___ SF1-lineage pituitary neuroendocrine tumor
  ___ Gonadotroph tumor
  ___ Other (specify): _____
___ Pituitary neuroendocrine tumor of no distinct cell lineage
  ___ Null cell tumor
  ___ Unusual plurihormonal (multilineage) pituitary neuroendocrine tumors (specify):
_____
  ___ Pituitary neuroendocrine tumor, NOS
___ Other (specify): _____
  +Histologic Type Comment: _____

Tumor Proliferative Activity (Note E)

Ki-67 Labeling Index#
 # The Ki-67 proliferation assessment should follow the IARC / WHO guidelines. Visual estimation based on routine microscopic examination (also known as eyeballing) is not allowed.
___ Specify Ki-67 percentage: _____ %
  Ki-67 Methodology
  ___ Manual count
  ___ Automated image analysis
  ___ Other (specify): _____
___ Pending (specify): _____
___ Cannot be determined (explain): _____

+Mitotic Count
___ Specify number of mitoses per 2 mm2: _____ mitoses per 2 mm2
___ Other (specify): _____

Histologically Confirmed Invasion (Note F)
___ Not identified
___ Present (specify): _____
___ Cannot be determined: _____

+Reticulin Histochemistry (Note G)
___ Not performed
___ Disrupted
___ Expanded acini
___ Other (specify): _____

+PAS Histochemistry (Note G)
___ Not performed
___ Positive
___ Negative
___ Other (specify): _____

Immunohistochemical Features (Note G)

TPIT
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

PIT1
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

SF1
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

+GATA3
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

+ER-alpha
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

+Alpha-subunit
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

ACTH (required only for TPIT lineage tumors)
___ Not applicable
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

GH (required only for PIT1 lineage tumors)
___ Not applicable
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

PRL (required only for PIT1 lineage tumors)
___ Not applicable
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

Beta-TSH (required only for PIT1 lineage tumors)
___ Not applicable
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

+Beta-FSH
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

+Beta-LH
___ Positive, diffuse
___ Positive, focal
___ Negative
___ Other (specify): _____

+p27
___ Intact
___ Lost
___ Other (specify): _____

+ATRX
___ Intact
___ Lost
___ Other (specify): _____

+p53
___ Abnormal
___ Wild-type
___ Other (specify): _____

Low Molecular Weight Keratins (required for PIT1 and TPIT lineage tumors, and those with no distinct cell lineages (e.g., null cell tumor))
___ Not applicable
___ Positive (specify, e.g., CAM5.2, CK8/18): _____
   Extent of Reactivity
   ___ Diffuse
   ___ Focal (non-diffuse)
   ___ Other (specify): _____
   Pattern of Reactivity (select all that apply)
   ___ Fibrous bodies (accounting for greater than 70% of the tumor)
   ___ Fibrous bodies (variable / scattered, accounting for less than 70% of the tumor)
   ___ Perinuclear cytoplasmic
   ___ Diffuse dense cytoplasmic

___ Ring-like cytoplasmic
___ Membranous
___ Other (specify): _____
___ Negative
___ Other (specify): _____

Other Markers (repeat this section for up to 10 markers)
    +Other Marker (specify): _____
        Specify Results: _____

Metastatic Site(s) (required only if applicable) (Note H) (select all that apply)
___ Not applicable
___ Cerebrospinal (specify site(s)): _____
___ Liver
___ Bone
___ Other (specify): _____

Non-tumorous Pituitary Site(s) (Note I) (select all that apply)
___ None identified
___ Adenohypophysis identified
    Crooke's Hyaline Change of the Non-tumorous Corticotrophs (required for all corticotroph tumors)
    ___ Not applicable
    ___ Present
    ___ Not identified
    ___ Other (specify): _____
    +Pituitary Hyperplasia
    ___ Present
    ___ Not identified
___ Neurohypophysis identified
___ Cannot be determined (explain): _____

ADDITIONAL FINDINGS

+Additional Findings (select all that apply)
___ None identified
___ Hypophysitis (specify): _____
___ Craniopharyngioma (specify type): _____
___ Rathke's cleft cyst (specify): _____
___ Acute hemorrhagic necrosis (apoplexy)
___ Other (specify): _____

COMMENTS

Comment(s): _____

Explanatory Notes

A. Scope of Guidelines

The reporting of pituitary neuroendocrine tumors is improved by the provision of a case summary that consolidates the clinical, biochemical, radiologic, morphologic and immunohistochemical features. The use of a structured report ensures completeness of the data required to achieve clinico-pathological correlations for a correct diagnosis and an appropriate treatment plan. This case summary attempts to remain simple while ensuring the collection of all relevant data in accordance with the World Health Organization (WHO) Classification of Tumors.[1,2] Patients with pituitary tumors may have hormone excess syndromes and these should be specified.[2,3,4] Unlike most other cancers, there is no TNM staging method approved as yet for sellar tumors; this remains a work in progress and will likely follow systems that have shown clinical utility for prognosis but have been misclassified as "grade" and are based on tumor size from imaging and extent of invasion. This protocol is to be used as a guide and resource, an adjunct to diagnosing and managing pituitary neuroendocrine tumors (PitNETs) in a standardized manner as per the 5th edition of the WHO classification of Endocrine and Neuroendocrine Tumors.[1,2] It should not be used as a substitute for dissection or grossing techniques and does not give histologic parameters to reach the diagnosis. Subjectivity is always a factor, and elements listed are not meant to be arbitrary but are meant to provide uniformity of reporting across all the disciplines that use the information. It is a foundation of practical information that will help to meet the requirements of daily practice to benefit both clinicians and patients alike.

References
1. WHO Classification of Tumours Editorial Board. *Endocrine and neuroendocrine tumours.* Lyon (France): International Agency for Research on Cancer; forthcoming. (WHO classification of tumours series, 5th ed.; vol. 10). https://publications.iarc.fr.
2. Asa SL, Mete O, Perry A, Osamura RY. Overview of the 2022 WHO Classification of Pituitary Tumors. *Endocr Pathol.* 2022 Mar;33(1):6-26.
3. Mete O, Asa SL. Clinicopathological correlations in pituitary adenomas. *Brain Pathol.* 2012 Jul;22(4):443-53.
4. Asa SL, Perry A. *Tumors of the Pituitary Gland.* Atlas of Tumor and Nontumor Pathology, Series 5, Fascicle 1. Arlington VA: ARP Press, 2020.

B. Specimen

Most pituitary tumors arise in the sella turcica and the surgical procedures include transsphenoidal approaches or transcranial resections. The approach is an indication of the clinical extent of disease and also plays a role in determining the type of specimen that will be obtained, since transsphenoidal surgeries usually result in fragmented small pieces of tissue. Rarely tumors can occur in ectopic locations[1,2,3] or can be diagnosed from their invasive fronts (e.g., sinonasal mucosa involvement),[3] or can be encountered in a teratoma,[4] and these should be noted. It is encouraged to document the size of the specimen to highlight the discordance with the tumor size since some specimens may be composed largely of non-tumorous parenchyma or may consist of a very limited amount of tumor; this may help to correlate with the tumor size on imaging. Because of the importance of immunohistochemistry in tumor classification, it is encouraged to document if the tissue was received fresh or in formalin for immunohistochemical biomarkers and also ultrastructure examination (rare tumors).

References

1. Mete O, Wenig BM. Update from the 5th Edition of the World Health Organization Classification of Head and Neck Tumors: Overview of the 2022 WHO Classification of Head and Neck Neuroendocrine Neoplasms. *Head Neck Pathol.* 2022 Mar;16(1):123-142.
2. Asa SL, Mete O, Perry A, Osamura RY. Overview of the 2022 WHO Classification of Pituitary Tumors. *Endocr Pathol.* 2022 Mar;33(1):6-26.
3. Hyrcza MD, Ezzat S, Mete O, Asa SL. Pituitary Adenomas Presenting as Sinonasal or Nasopharyngeal Masses: A Case Series Illustrating Potential Diagnostic Pitfalls. *Am J Surg Pathol.* 2017 Apr;41(4):525-534.
4. Hodgson A, Pakbaz S, Shenouda C, Francis JA, Mete O. Mixed Sparsely Granulated Lactotroph and Densely Granulated Somatotroph Pituitary Neuroendocrine Tumor Expands the Spectrum of Neuroendocrine Neoplasms in Ovarian Teratomas: The Role of Pituitary Neuroendocrine Cell Lineage Biomarkers. *Endocr Pathol.* 2020 Sep;31(3):315-319.

C. Tumor Number

Most pituitary tumors are solitary neoplasms but in about 1% of cases there may be multiple synchronous tumors in a single patient and usually in a single specimen.[1] It is important to consider this possibility when the immunohistochemical profile is unusual; the findings may represent two discrete lesions with different patterns of immunoreactivity.[1,2]

References

1. Mete O, Alshaikh OM, Cintosun A, Ezzat S, Asa SL. Synchronous Multiple Pituitary Neuroendocrine Tumors of Different Cell Lineages. *Endocr Pathol.* 2018 Dec;29(4):332-338.
2. Mete O, Asa SL. Structure, Function, and Morphology in the Classification of Pituitary Neuroendocrine Tumors: The Importance of Routine Analysis of Pituitary Transcription Factors. *Endocr Pathol.* 2020 Dec;31(4):330-336.

D. Histologic Tumor Types and Subtypes

The classification of PitNETs is based almost exclusively on the expression of transcription factors and hormones.[1,2,3,4] The WHO has endorsed a cytogenetic classification based on cell lineage.[1,5] Staining for PIT1, TPIT and SF1 is required to identify the appropriate lineage.[1,2,3,4,5,6] The addition of GATA3 and ERα stains allows a more accurate classification (Table 1). Staining for all of the known hormones is not required for TPIT and SF1 lineage tumors; however, within the PIT1-lineage tumors, the use of PIT1-lineage hormones (Growth hormone, Prolactin and Thyroid stimulating hormone) does enhance the ability to accurately classify individual PIT1-lineage tumors and facilitates the identification of tumor subtypes as well as uncommon tumors.[1,2] From such a perspective, the distribution of hormone positivity is important; some tumors (such as the immature PIT1-lineage tumor and acidophil stem cell tumor) are specifically recognized by focal positivity that contrasts with the more diffuse patterns seen in most mature adenohypophyseal neuroendocrine cells. Components of mixed somatotroph and lactotroph tumors should be subtyped using the WHO tumor subtyping (e.g., mixed sparsely granulated somatotroph tumor and sparsely granulated lactotroph tumor). Similarly, for multiple PitNETs of distinct cell lineages, one should subtype each tumor component (e.g., multiple PitNETs consisting of densely granulated corticotroph tumor and sparsely granulated lactotroph tumor).

Keratin staining is critical for most PitNETs. Although the use of keratin is strongly encouraged in all PitNETs, it is required in all PIT1 and TPIT lineage tumors[6] as well as in the setting of PitNETs with no distinct cell lineages (e.g., null cell tumors). The most widely used stain is CAM5.2 that is mimicked by

CK18, but some labs use AE1/AE3.[4,7] Some tumor types, such as sparsely granulated somatotroph tumors, are defined by their keratin pattern.[1,2] Classification of a null cell tumor is a diagnosis of exclusion from other neuroendocrine neoplasms (e.g., sellar paragangliomas [keratin-negative/non-epithelial neuroendocrine neoplasm of paraganglia] and metastatic neuroendocrine neoplasms) and requires lack of reactivity for the various transcription factors and hormones.[1,2,8,9] Thus, this section requires completion of Section G on Immunohistochemical Features.[10]

Table 1. Classification of Pituitary Neuroendocrine Tumors (PitNETs)

Transcription factor Family	Tumor type	Biomarkers
TPIT	Densely granulated corticotroph tumor†	TPIT, PAS (diffuse/strong), ACTH (diffuse/strong), keratins (diffuse cytoplasmic)
	Sparsely granulated corticotroph tumor†	TPIT, PAS (weak and can be focal), ACTH (weak and can be focal), keratins (diffuse cytoplasmic)
	Crooke cell tumor	TPIT, PAS (cell periphery and perinuclear), ACTH (cell periphery and perinuclear), keratins (ring-like)
PIT1	Densely granulated somatotroph tumor	PIT1, GH (diffuse/strong), alpha-subunit, keratins (perinuclear)
	Sparsely granulated somatotroph tumor	PIT1, GH (weak), keratins (fibrous bodies >70%)
	Densely granulated lactotroph tumor	PIT1, ER, PRL
	Sparsely granulated lactotroph tumor	PIT1, ER, PRL
	Mammosomatotroph tumor	PIT1, ER, GH (diffuse/strong), PRL (extent is often less than that of GH positivity), alpha-subunit, keratins (perinuclear)
	Thyrotroph tumor	PIT1, GATA3, TSH, alpha-subunit
	Mature plurihormonal PIT1-lineage tumor	PIT1, ER, GATA3, GH (diffuse/strong), PRL (variable), TSH (variable), alpha-subunit (variable), keratins (perinuclear)
	Immature PIT1-lineage tumor	PIT1 (diffuse) ± ER* ± GATA3** ± GH* ± PRL* ± TSH* ± alpha-subunit*, keratins
	Acidophil stem cell tumor	PIT1, ER, PRL, GH (weak), keratins (rare fibrous bodies***), alpha subunit (variable/focal)
SF1	Gonadotroph tumor	SF1, GATA3****, ER, FSH, LH, alpha-subunit
None	Null cell tumor	None (a diagnosis of exclusion from other neuroendocrine neoplasms)
Multiple	Multilineage PitNET (also known as plurihormonal tumor in the 2022 WHO classification)	Multiple variable
	Multiple synchronous PitNETs	Multiple variable but in distinct cell populations

TPIT: T-box transcription factor; PIT1: Pituitary transcription factor 1; SF1: Steroidogenic factor 1; ER: Estrogen receptor; PAS: Periodic Acid Shiff; ACTH: Adrenocorticotropic hormone; GH: Growth hormone; PRL: Prolactin; TSH: Thyroid stimulating hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; *non-diffuse variable (e.g., focal or patchy) reactivity; ** can be diffuse or focal; ***not a specific finding since rare fibrous bodies can occur in other PitNETs particularly in PIT1 lineage tumors; ****Alone GATA3 is not a diagnostic feature of gonadotroph tumor; † biochemically non-functional densely granulated corticotroph and sparsely granulated corticotroph tumors are known as silent corticotroph tumors type I and type II, respectively; however, silent corticotroph tumors are not histologic subtypes of TPIT-lineage PitNETs.

References

1. Asa SL, Mete O, Perry A, Osamura RY. Overview of the 2022 WHO Classification of Pituitary Tumors. *Endocr Pathol.* 2022 Mar;33(1):6-26.
2. Mete O, Asa SL. Structure, Function, and Morphology in the Classification of Pituitary Neuroendocrine Tumors: The Importance of Routine Analysis of Pituitary Transcription Factors. *Endocr Pathol.* 2020 Dec;31(4):330-336.
3. Asa SL, Mete O, Cusimano MD, McCutcheon IE, Perry A, Yamada S, Nishioka H, Casar-Borota O, Uccella S, La Rosa S, Grossman AB, Ezzat S; Attendees of the 15th Meeting of the International Pituitary Pathology Club, Istanbul October 2019. Pituitary neuroendocrine tumors: a model for neuroendocrine tumor classification. *Mod Pathol.* 2021 Sep;34(9):1634-1650.
4. Mete O, Cintosun A, Pressman I, Asa SL. Epidemiology and biomarker profile of pituitary adenohypophysial tumors. *Mod Pathol.* 2018 Jun;31(6):900-909.
5. WHO Classification of Tumours Editorial Board. *Endocrine and neuroendocrine tumours.* Lyon (France): International Agency for Research on Cancer; forthcoming. (WHO classification of tumours series, 5th ed.; vol. 10). https://publications.iarc.fr.
6. McDonald WC. Pituitary adenoma classification: Tools to improve the current system. *Free Neuropathol.* 2024 Jan 10; 5:5-2.
7. Asa SL, Mete O. Cytokeratin profiles in pituitary neuroendocrine tumors. *Hum Pathol.* 2021 Jan; 107:87-95.
8. Mete O, Wenig BM. Update from the 5th Edition of the World Health Organization Classification of Head and Neck Tumors: Overview of the 2022 WHO Classification of Head and Neck Neuroendocrine Neoplasms. *Head Neck Pathol.* 2022 Mar;16(1):123-142.
9. Mete O, Lopes MB. Overview of the 2017 WHO Classification of Pituitary Tumors. *Endocr Pathol.* 2017 Sep;28(3):228-243.
10. Asa SL, Mete O. Immunohistochemical Biomarkers in Pituitary Pathology. *Endocr Pathol.* 2018 Jun;29(2):130-136.

E. Tumor Proliferative Activity

Like other neuroendocrine neoplasms, clinical behavior and prognosis are determined in part by growth rate that is reflected in mitotic activity and Ki-67 labeling. Most of the published data are based on Ki-67 labeling, but documenting the mitotic count is also desirable. Similar to other neuroendocrine neoplasms, the mitotic count is reported as number of mitoses per 2mm^2, at least 10mm^2 evaluated in the most mitotically active part(s) of the tumor. The hot spot mitotic counting may apply contiguous round fields or random counting that uses a randomization method to avoid bias.[1] As specified by the WHO and based on accuracy studies, the Ki-67 assessment should be based on printout of a photo or an automated image analysis algorithm; eyeball estimates are not accurate or acceptable.[2] In general, selecting multiple small hot spots (at least 500 tumor cells) from different hot spot regions of the tumor rather than a single larger

area of the same tumor are recommended.[3] Unlike other epithelial well differentiated neuroendocrine neoplasms (neuroendocrine tumors), PitNETs are not graded based on Ki67 or mitoses since classification by cell type typically provides more useful information.[4]

References

1. Cree IA, Tan PH, Travis WD, Wesseling P, Yagi Y, White VA, Lokuhetty D, Scolyer RA. Counting mitoses: SI(ze) matters! *Mod Pathol.* 2021 Sep;34(9):1651-1657.
2. Cree IA. From Counting Mitoses to Ki67 Assessment: Technical Pitfalls in the New WHO Classification of Endocrine and Neuroendocrine Tumors. *Endocr Pathol.* 2022 Mar;33(1):3-5.
3. Volynskaya Z, Mete O, Pakbaz S, Al-Ghamdi D, Asa SL. Ki67 Quantitative Interpretation: Insights using Image Analysis. *J Pathol Inform.* 2019 Mar 8; 10:8.
4. Asa SL, Mete O, Perry A, Osamura RY. Overview of the 2022 WHO Classification of Pituitary Tumors. *Endocr Pathol.* 2022 Mar;33(1):6-26.

F. Histologically Confirmed Invasion

Although there is no accepted TNM staging of PitNETs, local invasion is a well-documented variable of prognostic significance.[1,2,3] It is important to include this information in the report when it is available. Invasion of dura (less clinically relevant), bone, respiratory (sinus) mucosa or brain tissue are the common sites of invasion identified in pathology specimens.

References

1. Knosp E, Steiner E, Kitz K, Matula C. Pituitary adenomas with invasion of the cavernous sinus space: a magnetic resonance imaging classification compared with surgical findings. *Neurosurgery.* 1993 Oct;33(4):610-7; discussion 617-8.
2. Raverot G, Ilie MD, Lasolle H, Amodru V, Trouillas J, Castinetti F, Brue T. Aggressive pituitary tumours and pituitary carcinomas. *Nat Rev Endocrinol.* 2021 Nov;17(11):671-684.
3. Trouillas J, Roy P, Sturm N, et al. A new prognostic clinicopathological classification of pituitary adenomas: a multicentric case-control study of 410 patients with 8 years post-operative follow-up. *Acta Neuropathol.* 2013 Jul;126(1):123-35.

G. Histochemical and Immunohistochemical Features

As indicated above in D, the classification of PitNETs is based on their immunoprofile.[1,2,3,4] The distinction of neoplasia from hyperplasia relies on reticulin staining that allows the identification of complete breakdown of acinar architecture in neoplasms, as opposed to the expanded but intact acini of hyperplasia.[3,5] The use of PAS in addition to ACTH is encouraged since it is often cleaner and easier to interpret. Staining for transcription factors and hormones is discussed above in D.

Additional stains that are helpful for PitNETs include p27 and ATRX[1,2,3,4,6]. Loss of p27 is reflective of clinical function by corticotroph tumors, since glucocorticoid excess suppresses p27 whereas clinically silent corticotroph tumors are usually associated with retained nuclear p27 staining.[4] ATRX loss and p53 alterations have been reported in aggressive PitNETs.[6,7]

References

1. Asa SL, Mete O, Perry A, Osamura RY. Overview of the 2022 WHO Classification of Pituitary Tumors. *Endocr Pathol.* 2022 Mar;33(1):6-26.

2. Mete O, Asa SL. Structure, Function, and Morphology in the Classification of Pituitary Neuroendocrine Tumors: The Importance of Routine Analysis of Pituitary Transcription Factors. *Endocr Pathol.* 2020 Dec;31(4):330-336.

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4. Mete O, Cintosun A, Pressman I, Asa SL. Epidemiology and biomarker profile of pituitary adenohypophysial tumors. *Mod Pathol.* 2018 Jun;31(6):900-909.

5. Mete O, Asa SL. Clinicopathological correlations in pituitary adenomas. *Brain Pathol.* 2012 Jul;22(4):443-53.

6. Casar-Borota O, Boldt HB, Engström BE, Andersen MS, Baussart B, Bengtsson D, Berinder K, Ekman B, Feldt-Rasmussen U, Höybye C, Jørgensen JOL, Kolnes AJ, Korbonits M, Rasmussen ÅK, Lindsay JR, Loughrey PB, Maiter D, Manojlovic-Gacic E, Pahnke J, Poliani PL, Popovic V, Ragnarsson O, Schalin-Jäntti C, Scheie D, Tóth M, Villa C, Wirenfeldt M, Kunicki J, Burman P. Corticotroph Aggressive Pituitary Tumors and Carcinomas Frequently Harbor ATRX Mutations. *J Clin Endocrinol Metab.* 2021 Mar 25;106(4):1183-1194.

7. Burman P, Casar-Borota O, Perez-Rivas LG, Dekkers OM. Aggressive Pituitary Tumors and Pituitary Carcinomas: From Pathology to Treatment. *J Clin Endocrinol Metab.* 2023 Jun 16;108(7):1585-1601. doi: 10.1210/clinem/dgad098. Erratum in: *J Clin Endocrinol Metab.* 2023 Sep 18;108(10):e1163.

H. Metastatic Site

Rarely aggressive PitNETs can metastasize.[1,2] The commonest sites are liver, bone and brain.[1] Spread through the CNS is discontinuous and this must be distinguished from local infiltration into the hypothalamus that does not count as metastasis.

References
1. Alshaikh OM, Asa SL, Mete O, Ezzat S. An Institutional Experience of Tumor Progression to Pituitary Carcinoma in a 15-Year Cohort of 1055 Consecutive Pituitary Neuroendocrine Tumors. *Endocr Pathol.* 2019 Jun;30(2):118-127.

2. Asa SL, Ezzat S. Pituitary carcinoma: reclassification and implications in the NET schema. *Endocr Oncol.* 2022 Mar 30;2(1): R14-R23.

I. Non-tumorous Pituitary

The presence of non-tumorous pituitary is an important feature to note for several reasons. Firstly, it confirms the location of the tumor and may indicate local invasion. Secondly, it provides internal positive controls for the various immunohistochemical biomarkers. Thirdly, it can provide useful information about the functional status of the patient. The identification of Crooke's hyaline change is a critical feature to document in patients with Cushing disease.[1,2,3,4] Other findings that may be associated with PitNETs include hypophysitis, craniopharyngioma, Rathke's cleft cyst or acute hemorrhagic necrosis (apoplexy).

References
1. Akirov A, Larouche V, Shimon I, Asa SL, Mete O, Sawka AM, Gentili F, Ezzat S. Significance of Crooke's Hyaline Change in Nontumorous Corticotrophs of Patients with Cushing Disease. *Front Endocrinol* (Lausanne). 2021 Mar 18; 12:620005.

2. Asa SL, Perry A. Tumors of the Pituitary Gland. *Atlas of Tumor and Nontumor Pathology*, Series 5, Fascicle 1. Arlington VA: ARP Press, 2020.

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4. Gomez-Hernandez K, Ezzat S, Asa SL, Mete Ö. Clinical Implications of Accurate Subtyping of Pituitary Adenomas: Perspectives from the Treating Physician. *Turk Patoloji Derg.* 2015;31 Suppl 1:4-17.