Protocol for the Examination of Specimens from Patients with Paraganglioma and Pheochromocytoma

Version: 1.0.0.0
Protocol Posting Date: March 2025

CAP Laboratory Accreditation Program Protocol Required Use Date: December 2025
The use of this protocol is required for accreditation purposes for both sympathetic and parasympathetic paragangliomas, pheochromocytomas, composite paragangliomas and composite pheochromocytomas.

For accreditation purposes, this protocol should be used for the following procedures AND tumor types:

Procedure	Description
Resection	Adrenalectomy and Extra-adrenal tumor (paraganglioma) excision
Tumor Type	**Description**
Tumors of the adrenal medulla and extra-adrenal paraganglia	Pheochromocytoma (adrenal paraganglioma), extra-adrenal paragangliomas from all anatomic sites, composite paraganglioma, composite pheochromocytoma

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

Tumor Type
Neuroblastic tumors
Peripheral nerve sheet tumors
Cauda equina neuroendocrine tumor (formerly known as cauda equine paraganglioma)
Composite gangliocytoma/neuroma and neuroendocrine tumor-COGNET (formerly known as gangliocytic paraganglioma)

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Glossary:
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

Synoptic reporting with core and conditional data elements for designated specimen types is required for accreditation.

- Data elements designated as core must be reported.
- Data elements designated as conditional only need to be reported if applicable.
- Data elements designated as optional are identified with "+". Although not required for accreditation, they may be considered for reporting.

This protocol is not required for recurrent or metastatic tumors resected at a different time than the primary tumor. This protocol is also not required for pathology reviews performed at a second institution (i.e., second opinion and referrals to another institution).

Full accreditation requirements can be found on the CAP website under Accreditation Checklists.

A list of core and conditional data elements can be found in the Summary of Required Elements under Resources on the CAP Cancer Protocols website.

Synoptic Reporting

All core and conditionally required data elements outlined on the surgical case summary from this cancer protocol must be displayed in synoptic report format. Synoptic format is defined as:

- Data element: followed by its answer (response), outline format without the paired Data element: Response format is NOT considered synoptic.
- The data element should be represented in the report as it is listed in the case summary. The response for any data element may be modified from those listed in the case summary, including "Cannot be determined" if appropriate.
- Each diagnostic parameter pair (Data element: Response) is listed on a separate line or in a tabular format to achieve visual separation. The following exceptions are allowed to be listed on one line:
    - Anatomic site or specimen, laterality, and procedure
    - Pathologic Stage Classification (pTNM) elements
    - Negative margins, as long as all negative margins are specifically enumerated where applicable
- The synoptic portion of the report can appear in the diagnosis section of the pathology report, at the end of the report or in a separate section, but all Data element: Responses must be listed together in one location
- Organizations and pathologists may choose to list the required elements in any order, use additional methods in order to enhance or achieve visual separation, or add optional items within the synoptic report. The report may have required elements in a summary format elsewhere in the report IN ADDITION TO but not as replacement for the synoptic report i.e., all required elements must be in the synoptic portion of the report in the format defined above.

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: (Paraganglioma and Pheochromocytoma)
Standard(s): AJCC 8

CLINICAL (Note A)

+Clinical History (select all that apply)
___ Family history of paraganglioma / pheochromocytoma or genetic syndrome (specify):
_____
___ Personal history of paraganglioma / pheochromocytoma or genetic syndrome (specify):
_____

Functional Status
___ Biochemically functioning
Select all that apply
___ Metanephrine and / or adrenaline
___ Normetanephrine and / or noradrenaline
___ 3-Methoxytyramine and / or dopamine
___ Other (specify): _____
___ Nonfunctional (biochemically non-functioning)
___ Biochemical analysis not performed
___ Not known (no information provided)
___ Other (specify): _____

+Tumor Scintigraphy or PET Avidity (select all that apply)
___ DOTATATE or DOTATOC PET
___ 123I-metaiodobenzylguanidine (MIBG) scintigraphy
___ Other (specify): _____

+Anatomic Location from Imaging (specify): _____

+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
Greatest Dimension of Second Dominant Tumor from Imaging Studies in Centimeters (cm) (required only if multifocal): _____ cm
___ Other (specify): _____
___ Not specified

SPECIMEN (Note B)

Procedure
___ Right adrenalectomy
___ Left adrenalectomy

___ Bilateral adrenalectomy
___ Adrenalectomy, NOS
___ Extra-adrenal excision (specify): _____
___ Other (specify): _____
___ Not specified

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

TUMOR (Notes C, D)

Tumor Number (Note C)
___ Unifocal
___ Multifocal
+Specify Number of Tumors: _____
___ Other (specify): _____

Tumor Location (Note C)
___ Intra-adrenal
___ Extra-adrenal (specify): _____
___ Other (specify): _____

+Tumor Size (Note C)
___ Greatest dimension in Centimeters (cm): _____ cm
+Additional Dimension in Centimeters (cm): ____ x ____ cm
Greatest Dimension of Second Dominant Tumor in Centimeters (cm) (required only if multifocal): _____ cm
___ Other (specify): _____
___ Not specified

Histologic Type(s) (Note C)
___ Paraganglioma
___ Pheochromocytoma (adrenal paraganglioma)
___ Micro-pheochromocytoma (subcentimeter adrenal paraganglioma)
___ Composite paraganglioma
___ Paraganglioma and ganglioneuroma, mature
___ Paraganglioma and ganglioneuroma, maturing
___ Paraganglioma and ganglioneuroblastoma, intermixed type
___ Paraganglioma and ganglioneuroblastoma, nodular type
___ Paraganglioma and neuroblastoma, undifferentiated type
___ Paraganglioma and neuroblastoma, poorly differentiated type
___ Paraganglioma and neuroblastoma, differentiating type
___ Paraganglioma and neuroblastic tumor, NOS
___ Paraganglioma and malignant peripheral nerve sheath tumor
___ Other (specify): _____

___ Composite pheochromocytoma
___ Pheochromocytoma and ganglioneuroma, mature
___ Pheochromocytoma and ganglioneuroma, maturing
___ Pheochromocytoma and ganglioneuroblastoma, intermixed type
___ Pheochromocytoma and ganglioneuroblastoma, nodular type
___ Pheochromocytoma and neuroblastoma, undifferentiated type
___ Pheochromocytoma and neuroblastoma, poorly differentiated type
___ Pheochromocytoma and neuroblastoma, differentiating type
___ Pheochromocytoma and neuroblastic tumor, NOS
___ Pheochromocytoma and malignant peripheral nerve sheath tumor
___ Other (specify): _____
___ Other histologic type not listed (specify): _____

Histologic Type(s) of Additional Tumors (required only if multiple tumors present) (select all that apply)

___ Not applicable (multiple tumors not present)
___ Paraganglioma
___ Pheochromocytoma (adrenal paraganglioma)
___ Micro-pheochromocytoma (subcentimeter adrenal paraganglioma)
___ Composite paraganglioma
___ Paraganglioma and ganglioneuroma, mature
___ Paraganglioma and ganglioneuroma, maturing
___ Paraganglioma and ganglioneuroblastoma, intermixed type
___ Paraganglioma and ganglioneuroblastoma, nodular type
___ Paraganglioma and neuroblastoma, undifferentiated type
___ Paraganglioma and neuroblastoma, poorly differentiated type
___ Paraganglioma and neuroblastoma, differentiating type
___ Paraganglioma and neuroblastic tumor, NOS
___ Paraganglioma and malignant peripheral nerve sheath tumor
___ Other (specify): _____
___ Composite pheochromocytoma
___ Pheochromocytoma and ganglioneuroma, mature
___ Pheochromocytoma and ganglioneuroma, maturing
___ Pheochromocytoma and ganglioneuroblastoma, intermixed type
___ Pheochromocytoma and ganglioneuroblastoma, nodular type
___ Pheochromocytoma and neuroblastoma, undifferentiated type
___ Pheochromocytoma and neuroblastoma, poorly differentiated type
___ Pheochromocytoma and neuroblastoma, differentiating type
___ Pheochromocytoma and neuroblastic tumor, NOS
___ Pheochromocytoma and malignant peripheral nerve sheath tumor
___ Other (specify): _____
___ Other histologic type not listed (specify): _____

+Histologic Type Comment: _____

Tumor Necrosis (Note C)

___ Not identified
___ Present

___ Other (specify): _____
___ Cannot be determined: _____

Angioinvasion (vascular invasion) (Note C)
___ Not identified
___ Present
___ Cannot be determined: _____

Lymphatic Invasion (Note C)
___ Not identified
___ Present
___ Cannot be determined: _____

Tumor Extent (Note C) (select all that apply)
___ Tumor confined to adrenal with no adrenal capsule invasion or no peritumoral soft tissue invasion
___ Adrenal capsule invasion without extra-adrenal tumor spread
___ Peri-adrenal or peri-tumoral soft tissue invasion
___ Adjacent organ / tissue invasion (e.g., liver, pancreas, spleen, kidney) (specify): _____
___ Other (specify): _____
___ Cannot be determined (explain): _____

Tumor Proliferative Activity (Note D)

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): _____

MARGINS

Margin Status
___ All margins negative for tumor
+Distance from Tumor to Closest Margin
Specify in Millimeters (mm)
___ Exact distance: _____ mm
___ At least: _____ mm
___ Other (specify): _____

___ Tumor present at margin
Margin(s) Involved by Tumor
___ Specify involved margin(s): _____
___ Cannot be determined (explain): _____
___ Other (specify): _____
___ Cannot be determined (explain): _____

+Margin Comment: _____

REGIONAL LYMPH NODES

Regional Lymph Node Status
___ Not applicable (no regional lymph nodes submitted or found)
___ Regional lymph nodes present
___ All regional lymph nodes negative for tumor
___ Tumor present in regional lymph node(s)
Number of Lymph Nodes with Tumor
___ Exact number (specify): _____
___ At least (specify): _____
___ Other (specify): _____
___ Cannot be determined (explain): _____
___ Other (specify): _____
___ Cannot be determined (explain): _____
Number of Lymph Nodes Examined
___ Exact number (specify): _____
___ At least (specify): _____
___ Other (specify): _____
___ Cannot be determined (explain): _____

+Regional Lymph Node Comment: _____

DISTANT METASTASIS (Note E)

Distant Site(s) Involved, if applicable (select all that apply)
___ Not applicable
___ Bone: _____
___ Liver: _____
___ Lung: _____
___ Other (specify): _____
___ Cannot be determined: _____

pTNM CLASSIFICATION (AJCC 8th Edition) (Note F)
Applied to all sympathetic paragangliomas and pheochromocytomas. Currently there is no defined TNM Classification for parasympathetic paragangliomas and composite paragangliomas / pheochromocytomas but users can apply the TNM staging below (based on the tumor size cutoff of 5 cm and the status of invasion) to facilitate uniform data collection.
Reporting of pT, pN, and (when applicable) pM categories is based on information available to the pathologist at the time the report is issued. As per the AJCC (Chapter 1, 8th Ed.) it is the managing physician's responsibility to establish the final pathologic stage based upon all pertinent information, including but potentially not limited to this pathology report.

Modified Classification (required only if applicable) (select all that apply)
___ Not applicable
___ y (post-neoadjuvant therapy)
___ r (recurrence)

pT Category
___ pT not assigned (cannot be determined based on available pathological information)
___ pT1: Pheochromocytoma less than 5 cm in greatest dimension, no extra-adrenal invasion
___ pT2: Pheochromocytoma greater than or equal to 5 cm or paraganglioma-sympathetic of any size, no extra-adrenal invasion
___ pT3: Tumor any size with invasion into surrounding organs / tissues (e.g., liver, pancreas, spleen, kidneys)

T Suffix (required only if applicable)
___ Not applicable
___ (m) multiple primary synchronous tumors in a single organ

pN Category
___ pN not assigned (no nodes submitted or found)
___ pN not assigned (cannot be determined based on available pathological information)
___ pN0: No lymph node metastasis
___ pN1: Regional lymph node metastasis

pM Category (required only if confirmed pathologically)
___ Not applicable - pM cannot be determined from the submitted specimen(s)
pM1: Distant metastasis
___ pM1a: Distant metastasis to only bone
___ pM1b: Distant metastasis to only distant lymph nodes / liver or lung
___ pM1c: Distant metastasis to bone plus multiple other sites
___ pM1 (subcategory cannot be determined)

ADDITIONAL FINDINGS (Note G)

Adrenal Medullary Hyperplasia (required for all adrenalectomy specimens)
___ Not applicable
___ Present
___ Absent
___ Other (specify): _____
___ Cannot be determined (explain): _____

+Additional Findings (select all that apply)
___ None identified
___ Other tumors (specify): _____
___ Other (specify): _____

SPECIAL STUDIES (Note H)

+Keratins (e.g., low molecular weight keratin, AE1 / AE3)
___ Positive (specify): _____
___ Negative

+Chromogranin-A
___ Positive
___ Negative

+Tyrosine Hydroxylase
___ Positive
___ Negative

+GATA3
___ Positive
___ Negative

+S100 and / or SOX10-positive Sustentacular Cell Network
___ Present
___ Not identified
___ Other (specify): _____

+SDHB
___ Loss (deficient)
___ Retained (normal staining)
___ Other (specify): _____

+Alpha-inhibin
___ Positive
___ Negative

+CAIX
___ Positive
___ Negative

+FH
___ Loss (deficient)
___ Retained (normal staining)
___ Other (specify): _____

+2-SC
___ Positive
___ Negative

+ATRX
___ Loss (deficient)
___ Retained (normal staining)
___ Other (specify): _____

Other Markers (repeat this section for up to 10 markers)

+Other Marker (specify): _____
Specify Results: _____

COMMENTS

Comment(s): _____

Explanatory Notes

A. Clinical

Paraganglia are neural crest-derived neuroendocrine organs that produce predominantly catecholamines (dopamine, norepinephrine and epinephrine).[1,2,3] Paraganglia are typically divided into two groups based on parasympathetic or sympathetic nervous system origin. Sympathetic paraganglia are further divided into two subgroups: the adrenal medulla, so-called “sympathoadrenal paraganglia” and extra-adrenal sympathetic paraganglia.[1,2,3] The anatomic site impacts the nomenclature of tumors arising from paraganglia; while tumors arising from the adrenal medulla are termed “pheochromocytomas or adrenal paragangliomas”, tumors arising from extra-adrenal locations are called “paragangliomas (PGLs)” regardless of their sympathetic or parasympathetic origins.[1,2,3,4] Pheochromocytomas and paragangliomas are collectively referred to PPGLs in the medical literature.

The etiology of most sporadic PGLs including pheochromocytomas is not known. However, it is now well-recognized that at least 40% of these tumors in adults are hereditary.[1,2,3,4,5,6,7] caused by germline variants of at least 23 genes. Hereditary pheochromocytomas and extra-adrenal PGLs (PPGLs) arising in patients with different genotypes have characteristic distributions and biochemical profiles and different likelihoods of metastasis.[1,2,3,4,5,6] In addition, a widening spectrum of associated tumors — including but not limited to gastrointestinal stromal tumors, renal cell carcinomas, and pituitary neuroendocrine tumors — are associated with multiple hereditary tumor syndromes. Discoveries of new susceptibility genes and genotype-phenotype correlations have led to the realization that individual patient care requires a complete integration of clinical, genetic, biochemical, imaging, and pathology findings. [2] While most patients lack family history, the identification of personal or family history of PPGL is often regarded a sign of hereditary disease in these neoplasms.

While pheochromocytomas and the majority of sympathetic PGLs are often associated with clinical symptoms, only a small percentage of parasympathetic PGLs are clinically symptomatic.[1,8] The functional status of a PPGL should be based on the biochemical status of the tumor. Specific genotype-biochemical correlations highlight the importance of laboratory testing to characterize patterns of catecholamine excess. Since catecholamines (dopamine, norepinephrine, and epinephrine) are not continuously secreted in normal physiology, biochemical testing for the O-methylated metabolites of dopamine, norepinephrine and epinephrine (3-methoxytyramine, normetanephrine and metanephrine, respectively) in plasma and/or urine is superior to measurement of the parent catecholamines.[3]

Progress in molecular biology has established genotype-phenotype correlations in PPGLs with respect to anatomic tumor distribution (e.g., intra-adrenal vs extra-adrenal), catecholamine production and risk of metastasis.[2,3,4] For instance, functional *VHL*-driven PPGLs tend to be associated with norepinephrine excess whereas functional *SDHx*-related PPGLs can be associated with either mixed dopamine and norepinephrine or dopamine excess.[1,2,3,4,9]. *RET*-, *NF1*-, *TMEM127*- and *MAX*-related PPLs are associated with epinephrine production.[1,2,3,4] Moreover, the risk of malignancy is increased in tumors with immature secretory phenotype characterized by the absence of epinephrine excess (e.g., *SDHB*-related PPGLs). *SDHB*-related tumors are more frequently observed in extra-adrenal locations and can reach larger tumor sizes with much lower tissue concentrations of catecholamines than other PGLs.[3] These data are of clinical significance in that integration of the biochemical profile with other information, such as tumor location and dimensions, becomes an important part of comprehensive synoptic reporting. Similar to epithelial

neuroendocrine neoplasms, PPGLs can produce and secrete other peptides that occasionally cause clinical hormone excess syndromes.[8,10]

Somatostatin receptor-based functional imaging techniques have revolutionized our ability to image neuroendocrine neoplasms including PPGLs. Functional imaging with radiotracers such as 123/131I-metaiodobenzylguanidine (123/131I-MIBG) have long been recognized as integral components in the diagnostic and therapeutic planning of patients with PPGLs.[11] Subsequently, the reduced sensitivity in extra-adrenal PGL and metastatic forms of the disease prompted investigations of other tracers. Gallium 68 (68GA)-DOTATATE PET/CT, which received FDA approval in 2016, has emerged as the most commonly used functional imaging radiotracer for detection of SSTR positive disease.[12] More recently in 2020, the FDA approved Copper DOTATATE PET (64Cu) imaging for these lesions. In a prospective head-to-head comparison between [64Cu]Cu-DOTATATE and [68Ga]Ga-DOTATOC PET/CT, Johnbeck et al. reported a slightly higher detection rate for the former (99.1% vs. 95.6%), with 701 concordant lesions on both scans.[13] The integration of functional imaging data is of clinical interest to ensure completeness of the synoptic report since it provides staging, prognostic, and potentially theranostic information in tumors that over-express SSTR2.

References

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B. Specimen

The procedure used to resect a tumor is based on anatomic locations/organs (e.g., adrenalectomy for pheochromocytomas/adrenal PGLs). In some cases, PPGLs are resected laparoscopically resulting in morcellation/extensive fragmentation that can compromise the ability to determine completeness of surgical resection and microscopic assessment of the invasive growth.

C. Tumor

The issue of tumor multifocality is very important and should be included in the synoptic report. Patients with multiple PPGLs should be investigated for the possibility of underlying genetic susceptibility and thus genetic testing is considered in the setting of multifocal PPGL.[1,2,3] Multifocal PPGL include multiple pheochromocytomas including their subcentimeter counterparts (also known as micro-pheochromocytoma) or a combination of an extra-adrenal PGL with a pheochromocytoma or multifocal extra-adrenal PGLs.

The anatomic location(s) of the tumor must be provided to the pathologist and be clearly documented in the synoptic report with the appropriate classification based on location. Similar to other guidelines, tumor size is a required field in surgical pathology reports. This information is required for tumor staging (see note F). Although the tumor size is not a universally accepted independent prognostic parameter in PPGLs, it is a component of the dynamic risk stratification since larger PPGLs (>5-7 cm) may be associated with an increased risk of metastasis..[1,2,3,4,5]

The concept of "benign" or "malignant" PPGL has been abandoned since the 2017 WHO classification of endocrine tumors. PPGLs are malignant non-epithelial neuroendocrine neoplasms originating from paraganglia.[1,2,3,4,5,6].Similar to other epithelial NENs, the term "metastatic PGL or pheochromocytoma" should be used in the setting of metastatic disease.[3] PPGLs are associated with varying risk of metastatic spread without any clear-cut morphological harbingers. The 2022 WHO classification of PPGLs does not endorse the use of multifactorial scoring systems (e.g., PASS, GAPP, modified GAPP, COPPS)[1,5,7] since all PPGLs are malignant neoplasms and PPGLs with higher scores may lead to overestimation of risk for metastasis, particularly in some subgroups.[7]

The current histological classification of PPGLs (Table 1) follow the 2022 WHO classification endocrine and neuroendocrine neoplasms[7]. In the 2022 WHO classification, the tumor formerly known as cauda equina PGL is now classified as cauda equina neuroendocrine tumor and the neoplasm known as gangliocytic paraganglioma has been renamed composite gangliocytoma/neuroma and neuroendocrine tumor (CoGNET).[1,7,8,9,10] These unusual epithelial NENs are not PPGLs and should not be reported with this worksheet.[1,7,8,9]

The rare composite PPGLs are diagnosed when a typical PPGL is combined with developmentally-related distinct tumor component including ganglioneuroma (mature or maturing), ganglioneuroblastoma (nodular or intermixed), neuroblastoma (undifferentiated, poorly differentiated or differentiating) or malignant peripheral nerve sheath tumors.[1,3,4,7] Scattered ganglion-like cells or neuropil-like degenerated stroma seen in PPGLs should not be misinterpreted as a component of a composite tumor. The composite elements are diagnosed and subtyped using universally defined histomorphology criteria (International Neuroblastoma Pathology Committee-INPC)[11] and/or biomarker features.[7] In the 2022 WHO classification, a minimum cut-off of 5% has been proposed for each tumor element to qualify as a composite PPGL; however, this would require adequate/thorough tumor sampling in an attempt to capture intra-tumoral heterogeneity.[1,7] Composite PPGLs are not well studied and most prognostic variables are less recognized. At this time, diagnosticians can also document the extent of composite elements and mitotic-karyorrhectic index (MKI) in neuroblastic elements of composite PPGLs. Composite PPGLs may occur in the setting of pathogenic *RET*, *MAX* and *NF1* variants.[1,12]

Irrespective of the anatomic location, PPGLs show overlapping cytomorphologic features. They display a variety of growth patterns. Sympathetic PPGLs often consist of polygonal cells (chromaffin cells) with amphophilic to basophilic cytoplasm, whereas parasympathetic PGLs consist of polygonal cells (chief cells) with relatively clearer cytoplasm than their sympathetic counterparts. Genotype-phenotype correlations underscored that VHL-related PPGLs contain usually a thick vascular capsule, hyalinized vascular and myxoid stroma, round tumor cells intermingled with small vessels, oncocytic cells, cells with predominantly amphophilic and clear cell cytoplasm, absence of intracytoplasmic hyaline globules and lipid degeneration.[1,2,7,13] *SDHx*-related PPGLs are often composed of tumor cells with variable intra-cytoplasmic vacuoles and/or oncocytic change.[1,2,6,14]

Expanded large confluent nests with central comedo necrosis have been described in a subset of metastastic PPGLs.[3] Degenerative changes should not be mistaken as tumor necrosis. Vascular and lymphatic invasion are critical elements in the dynamic risk stratification of PPGLs.[1,3,5] Vascular invasion in endocrine neoplasia requires the documentation of tumor cells invading through a vessel wall and forming intravascular tumor-platelet/thrombus complex or intravascular tumor cells admixed with platelets-thrombus.[6] In cases with subtle findings, CD61 immunohistochemistry can be used to highlight platelets admixed with intravascular tumor cells.

The 8th edition AJCC TNM criteria[15] also requires diagnosticians to document the text of histologically confirmed invasion into adjacent tissues/structures and organs. Therefore, local invasion into surrounding organs should be clearly documented to facilitate the staging. In addition, the extent of invasiveness of these tumors may affect the ability to obtain a surgical cure.[16] In morcellated or fragmented resection specimens, the status and extent of invasive growth and the completeness of the tumor excision cannot be assessed.

Table 1. Classification of Pheochromocytomas and Extra-Adrenal Paragangliomas

___Paraganglioma
___Pheochromocytoma (adrenal paraganglioma)
___Micro-pheochromocytoma (subcentimeter adrenal paraganglioma)
___Composite paraganglioma
  ___Paraganglioma and Ganglioneuroma, mature
  ___Paraganglioma and Ganglioneuroma, maturing
  ___Paraganglioma and Ganglioneuroblastoma, intermixed type
  ___ Paraganglioma and Ganglioneuroblastoma, nodular type
  ___Paraganglioma and Neuroblastoma, undifferentiated type
  ___Paraganglioma and Neuroblastoma, poorly differentiated type
  ___Paraganglioma and Neuroblastoma, differentiating type
  ___Paraganglioma and Neuroblastic tumor, NOS
  ___Paraganglioma and Malignant peripheral nerve sheath tumor
  ___Other (specify): _____
___Composite pheochromocytoma
  ___Pheochromocytoma and Ganglioneuroma, mature
  ___Pheochromocytoma and Ganglioneuroma, maturing
  ___Pheochromocytoma and Ganglioneuroblastoma, intermixed type
  ___Pheochromocytoma and Ganglioneuroblastoma, nodular type
  ___Pheochromocytoma and Neuroblastoma, undifferentiated type
  ___Pheochromocytoma and Neuroblastoma, poorly differentiated type
  ___Pheochromocytoma and Neuroblastoma, differentiating type
  ___Pheochromocytoma and Neuroblastic tumor, NOS
  ___Pheochromocytoma and Malignant peripheral nerve sheath tumor
  ___Other (specify): _____

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D. Tumor Proliferative Activity

Like epithelial NENs, clinical behavior and prognosis are determined in part by growth rate that is reflected in Ki-67 labeling and mitotic count.[1] The value of Ki-67 in tumor-related progression and metastasis has also been confirmed in the TCGA data.[2] Although there is no formal WHO grading for PPGLs, increased proliferative activity is an important variable in the dynamic risk stratification of PPGLs.[1,3,4]

As specified by the 2022 WHO classification and based on accuracy studies, the Ki-67 assessment from hot spots should be based on printout of a photo or an automated image analysis algorithm; eyeball estimates are not accurate or acceptable.[5,6] Similar to epithelial NENs, PPGLs can show intra-tumoral proliferative heterogeneity. 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 often recommended.[7] In the 2022 WHO classification proliferation in general is regarded as a dynamic variable rather than a static threshold, therefore, a precise numeric percentage or count should be provided for Ki67 labeling index or mitotic count of all neuroendocrine neoplasms.[6]

The mitotic rate should be 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.[8] The documentation of the methodology used to count mitosis is also encouraged. Only clearly identifiable mitotic figures should be counted;

hyperchromatic, karyorrhectic, or apoptotic nuclei should not be counted. The application of phosphoHistone-H3 immunohistochemistry can also be used to count mitotic figures.[5,6]. Because of variations in field size, the number of high-power fields (HPF) (at 40X magnification) for 10mm^2 (thereby 2mm^2) must be determined for each microscope (Table 2).

Table 2. Number of HPF Required for 10mm^2 and 2mm^2 Using Microscopes with Different Field Diameter

Field Diameter (mm)	Area (mm)	Number of HPF for 10 mm	Number of HPF for 2 mm
0.40	0.125	80	16
0.41	0.132	75	15
0.42	0.139	70	14
0.43	0.145	69	14
0.44	0.152	65	13
0.45	0.159	63	13
0.46	0.166	60	12
0.47	0.173	58	12
0.48	0.181	55	11
0.49	0.189	53	11
0.50	0.196	50	10
0.51	0.204	49	10
0.52	0.212	47	9
0.53	0.221	45	9
0.54	0.229	44	9
0.55	0.238	42	8
0.56	0.246	41	8
0.57	0.255	39	8
0.58	0.264	38	8
0.59	0.273	37	7
0.60	0.283	35	7
0.61	0.292	34	7
0.62	0.302	33	7
0.63	0.312	32	6
0.64	0.322	31	6
0.65	0.332	30	6
0.66	0.342	29	6
0.67	0.353	28	6
0.68	0.363	28	6
0.69	0.374	28	5

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E. Distant Metastases

Metastases are typically defined as PGLs in locations where normally paraganglia do not exist.[1] The 2022 WHO classification of PPGLs has addressed to concerns on the distinction of metastases.[1] Knowledge of the distribution of the autonomous nervous system and recognition of germline disease related multifocal PPGLs have improved our distinction of metastatic disease from multifocal disease in anatomic locations that have been traditionally considered as a metastatic site (e.g., liver, lung).[1,2] Lymph node and bone are the only reliable sites for unequivocal evidence of metastasis.[1,2]

Primary PGLs can occur in several organs including but not limited to lung, liver, prostate, kidney, urinary bladder, heart, mesentery.[1,2,3] However, without the knowledge on germline status of a patient, the distinction of multifocal primary PPGLs from metastatic disease can be difficult. The status of intratumoral sustentacular cell network has now been recognized as a useful tool to facilitate this distinction.[1] S100 and SOX10-positive sustentacular cells are thought to represent the non-neoplastic cellular component of PPGLs,[4] and metastases have been associated with the lack of sustentacular cell network.[5]

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F. pTNM Classification

The 8th edition of the TNM staging system of the American Joint Committee on Cancer (AJCC) is now recommended for staging sympathetic PGLs and pheochromocytomas.[1] Accordingly, TNM staging is based on size, location and invasiveness of the primary tumor, the presence or absence of locoregional metastases, and both the presence and site of distant metastases. Bone metastases alone are associated with longer survival than metastases to lung or liver. Currently there is no TNM classification for parasympathetic PGLs and composite PPGLs; however, the authors of this protocol encourage all users to apply the current template (based on the tumor size cutoff of 5 cm and the status of invasion) to facilitate uniform data collection.

According to AJCC/UICC convention, the designation "T" refers to a primary tumor that has not been previously treated. The symbol "p" refers to the pathologic classification of the TNM, as opposed to the clinical classification, and is based on gross and microscopic examination. pT entails a resection of the primary tumor or biopsy adequate to evaluate the highest pT category, pN entails removal of nodes adequate to validate lymph node metastasis, and pM implies microscopic examination of distant lesions. Clinical classification (cTNM) is usually carried out by the referring physician before treatment during initial evaluation of the patient or when pathologic classification is not possible.

Pathologic staging is usually performed after surgical resection of the primary tumor. Pathologic staging depends on pathologic documentation of the anatomic extent of disease, whether or not the primary tumor has been completely removed. If a biopsied tumor is not resected for any reason (e.g., when technically infeasible) and if the highest T and N categories or the M1 category of the tumor can be confirmed microscopically, the criteria for pathologic classification and staging have been satisfied without total removal of the primary cancer.

TNM Descriptors

For identification of special cases of TNM or pTNM classifications, the "m" suffix and "y," "r," and "a" prefixes are used. Although they do not affect the stage grouping, they indicate cases needing separate analysis.

The "m" suffix indicates the presence of multiple primary tumors in a single site and is recorded in parentheses: pT(m)NM.

The "y" prefix indicates those cases in which classification is performed during or after initial multimodality therapy (i.e., neoadjuvant chemotherapy, radiation therapy, or both chemotherapy and radiation therapy). The cTNM or pTNM category is identified by a "y" prefix. The ycTNM or ypTNM categorizes the extent of tumor actually present at the time of that examination. The "y" categorization is not an estimate of tumor before multimodality therapy (i.e., before initiation of neoadjuvant therapy).

The "r" prefix indicates a recurrent tumor when staged after a documented disease-free interval and is identified by the "r" prefix: rTNM.

The "a" prefix designates the stage determined at autopsy: aTNM.

T Category Considerations

The "m" designation applies only to grossly recognizable, synchronous primary tumors and not to a single, grossly detected tumor with multiple separate microscopic foci. If more than 1 tumor is present, the tumor with the highest T category should be classified according to the pT definitions, and either the multiplicity

("m") or the actual number of simultaneous multiple tumors (e.g., "3") should be indicated in parentheses after the T category of the primary tumor (e.g., pT3[m] or pT3[2]).

Four stage groups are compiled from the TNM data:

TNM staging

Tumor (pT)

- TX: Primary tumor cannot be assessed
- T1: Pheochromocytoma less than 5 cm in greatest dimension, no extra-adrenal invasion
- T2: Pheochromocytoma greater than or equal to 5 cm or sympathetic paraganglioma of any size, no extra-adrenal invasion
- T3: Tumor any size with invasion into surrounding organs/tissues (e.g., liver, pancreas, spleen, kidneys)

Regional lymph nodes

- N0: Regional lymph nodes cannot be assessed
- N0: No lymph node metastasis
- N1: Regional lymph node metastasis

Distant metastasis

- M0: No distant metastasis
- M1a: Distant metastasis to only bone
- M1b: Distant metastasis to only distant lymph nodes/liver or lung
- M1c: Distant metastasis to bone plus multiple other sites

Stage groups

- Stage 1: T1, N0, M0
- Stage 2: T2, N0, M0
- Stage 3: T1, N1, M0 or T2, N1, M0 or T3, any N, M0
- Stage 4: any T, any N, M1

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G. Additional Findings

Diffuse (non-nodular) expansion of adrenal medulla, which expands to comprise more than one-third of the adrenal gland thickness, qualifies for adrenal medullary hyperplasia that is a precursor lesion for inherited PPGLs in association with pathogenic *RET* (most common), *MAX*, *TMEM127*, and *NF1* variants.[1,2,3,4,5,6,7] Diagnosticians should also remember that adrenal medulla is normally present only in the head and body of the adrenal gland, but not in the tail of the gland with only minimal extension into the alae. So an abnormal location of the medulla (e.g., extension of the adrenal medulla into the tail) also warrants a diagnosis of diffuse adrenal medullary hyperplasia.[2,3,8] Therefore, the non-tumorous adrenal gland should be sampled in toto. Irrespective of the size, any distinct nodular proliferation of chromaffin cell origin in the medulla would warrant a diagnosis of micro-pheochromocytoma.[1,3] In the setting of diffuse compact cell

adrenal cortical hyperplasia, the possibility of ectopic ACTH/CRH-production from a pheochromocytoma should be considered.[8]

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H. Special Studies

The application of routine immunohistochemical biomarkers is essential in the field of NENs including PPGLs. In the 2022 WHO classification of NENs, testing of keratin expression status is recommended for all NENs.[1,2,3,4] While negativity alone does not exclude an epithelial NENs, PPGLs are typically negative for keratins. Chromogranin A is the classical biomarker to confirm neuroendocrine differentiation, and INSM1 is also useful in the appropriate morphological setting.[1,2,3,4] The demonstration of intratumoral sustentacular cell network is not specific for PPGLs and can be seen in epithelial NENs.[1,2] Positivity for GATA3 is commonly used to confirm the paraganglia origin in keratin-negative NENs; however, GATA3 is also expressed in other NENs which can sometimes be keratin-negative (e.g., gonadotroph tumor). There are also other less frequently adopted markers including but not limited to PHOX2B[5] and they have been shown to support the paraganglia origin, thus a PPGL diagnosis in the setting of a keratin-negative NEN.[6,7] PHOX2B is also used in the distinction of ganglioneuronal elements of composite tumors. S100 and SOX10 can be used to highlight schwannian stroma in composite tumors.[1] Tyrosine hydroxylase (the rate limiting enzyme in the synthesis of catecholamines) is typically positive in most functional PPGLs.[1,2,3,4] However, immunohistochemical biomarkers should be used in a panel as positivity of a single marker may be misleading. Parasympathetic PGLs can have absent to reduced expression for chromogranin-A and tyrosine hydroxylase. The demonstration of intratumoral sustentacular cell network can facilitate the distinction of multifocal primary PPGLs from metastatic disease as discussed on note E. More importantly, reduced intra-tumoral sustentacular cell network in a primary PPGL is of interest as an

emerging variable in the dynamic risk stratification of PPGLs.[1,2] Loss of ATRX and increased MIB1 (Ki-67) labeling index (often >3%) are also adverse parameters in the dynamic risk stratification.[1,2,4,7]

The highly heritable nature of PPGLs and lack of family history in most affected patients have resulted in reflex biomarker testing regarding their pathogenesis. The desirable molecular immunohistochemistry tools include SDHB, alpha-inhibin, CAIX, FH and 2SC.[1] Global loss of cytoplasmic SDHB expression is referred to SDH deficiency and can be associated with any *SDHx* alterations.[1] Positivity for alpha-inhibin can occur in PGLs and pheochromocytomas that are associated with pseudohypoxia pathway (e.g., *SDHx*- and *VHL*-related PPGLs) that manifest with dopaminergic or mixed dopaminergic and adrenergic tumors as well as in the setting of pure noradrenergic immature secretory phenotype.[1,7] Membranous CAIX is also regarded a feature of *VHL*-related PPGLs.[1,7,8,9] *FH*-related pathogenesis can be screened by loss of FH (fumarate hydratase) and positivity for 2-SC (2-succinocystein).[1,10] If available, menin can be used to screen for MEN1-related pathogenesis.[1,2,11]The current experiences with MAX antibody (MEN5-related manifestation) are conflicting among experts. BAP1 immunohistochemistry is also unreliable.[12] Although immunohistochemical biomarkers are very helpful in raising the possibility of heritable disease and/or underlying pathogenesis, the gold standard is germline genetic testing which should be offered in all patients with PPGLs.

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