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Protocol for the Examination of Specimens from Patients with Pheochromocytomas and Extra-adrenal Paragangliomas

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Abstract

During the last decade there have been revolutionary breakthroughs in understanding the biology of pheochromocytomas and extra-adrenal paragangliomas. Discoveries of new susceptibility genes and genotype-phenotype correlations have led to the realization that appropriate patient care requires a complete integration of clinical, genetic, biochemical, imaging, and pathology findings. Clinical practice has in many cases not kept pace with the rate of discovery, underscoring a need for updated procedures for evaluation of patient specimens and reporting of data. We therefore propose a new synoptic reporting approach for pheochromocytomas and extra-adrenal paragangliomas that will provide clear and uniform information to pathologists and clinicians, in order to advance the diagnosis of these neoplasms and optimize patient care.

Keywords

Paraganglioma; pheochromocytoma; metastatic paraganglioma; metastatic pheochromocytoma; catecholamine; genotype-phenotype correlations; synoptic reporting

INTRODUCTION

During the last decade there have been revolutionary breakthroughs in understanding the biology of pheochromocytomas and extra-adrenal paragangliomas. It is now recognized that at least 30% of these tumors are hereditary, caused by germline mutations of at least 10 genes [1-9]. Hereditary pheochromocytomas and extra-adrenal paragangliomas arising in patients with different genotypes have characteristic distributions and biochemical profiles and different likelihoods of metastasis [6-9]. In addition, a widening spectrum of associated tumors — including gastrointestinal stromal tumors, renal cell carcinomas, and pituitary

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adenomas — is associated with newly discovered hereditary tumor syndromes. Discoveries of new susceptibility genes and genotype-phenotype correlations have led to the realization that appropriate patient care requires a complete integration of clinical, genetic, biochemical, imaging, and pathology findings [6-9]. There is a corresponding need for updates in clinical practice to incorporate these recent discoveries. We therefore propose a new synoptic reporting approach for pheochromocytomas and extra-adrenal paragangliomas that will provide clear and uniform information to pathologists and clinicians, in order to advance the diagnosis of these neoplasms and optimize patient care.

SCOPE OF GUIDELINES

Beyond differential diagnosis, pathologists play important roles in identifying clues to hereditary disease and alerting clinicians to possible associated lesions and their significance. The proposed checklist aims to provide uniform and complete data to allow thorough evaluation of pheochromocytomas and extra-adrenal paragangliomas. This checklist will guide pathologists to issue standardized reports. It does not include the detailed information required to reach the diagnosis of pheochromocytoma or extra-adrenal paraganglioma; that is provided elsewhere [1-5]. A novel component of the checklist is a formatted clinicopathologic correlation.

PATHOLOGY CASE SUMMARY (CHECKLIST)

Select a Single Response Unless Otherwise Indicated—† Data elements marked with this are not required. While they are important, some are not yet validated or regularly used in a patient management and others may not be readily available to the Pathologist examining the specimen.

Procedure (select all that apply) (note A)

___Adrenalectomy

___Right

___Left

___Bilateral

___Extra-adrenal excision (specify): _____

___Other (specify): _____

___Not specified

†Biochemical Features (select all that apply) (note B)

___Biochemically Functioning

__Metanephrine and/or adrenaline

__Normetanephrine and/or noradrenaline

__Methoxytyramine and/or dopamine

__Other (specify): _____

___Biochemically silent

___Biochemical analysis not performed

___Cannot be determined

†Tumor Scintigraphy or Positron Emission Tomography (PET) (select all that apply) (note C)

___^{123}I-metaiodobenzylguanidine scintigraphy

___^{18}F-6-fluorodopamine PET

___^{18}F-6-fluorodihydroxphenylalanine PET

___^{18}F-fluorodeoxyglucose PET

___Other (specify): _____

† **Tumor Location and Size (from imaging) (note D)**

Anatomic location (specify): _____

Greatest dimension: ___ cm

†Additional dimensions: ___ × ___ cm

Second dominant tumor if multifocal

___× ____× ____cm

†Additional dimensions if more than 2 foci: _____

___Cannot be determined

†**Received**:

___Fresh

___In formalin

†Fixation time: _____

___Other (specify): _____

† **Specimen Integrity**

___Intact

___Fragmented

Specimen Size

___× ____× ____cm

†Additional dimensions: _____

† **Specimen Weight**

___grams

Tumor Focality

__Unifocal or

__Multifocal (specify #): _____

__Cannot be determined

Tumor Size

Dominant tumor

___× ____× ____cm

Second dominant tumor if multifocal

___× ____× ____cm

†Additional dimensions if more than 2 foci: _____

Tumor Type (note E)

___Pheochromocytoma(s) specify site (s):_____

___Extra-adrenal paraganglioma(s) specify site(s):_____

___Composite pheochromocytoma (specify):_____

___Composite paraganglioma (specify site and components):_____

___Gangliocytic paraganglioma

___Metastatic pheochromocytoma, specify site: _____

___Metastatic paraganglioma, specify site: _____

___Other (specify):_____

Histologic Features (Note F)

Growth pattern (select all that apply)

___Nested (alveolar, zellballen) pattern

___Trabecular pattern

___Diffuse (solid) pattern

___Expanded large confluent nests

___Other (specify): _____

Composite tumor elements (select all that apply)

___Absent

___Present (select all that apply):

___Neuroblastoma

Specify extent (%):

Degree of differentiation of the neuroblastic component (select all that apply)

___Undifferentiated

___Poorly differentiated

___Differentiating

___Cannot be assessed

___Ganglioneuroblastoma

Specify extent (%):

Subtypes:

___Nodular subtype

Specify number of nodules: _____

Specify the degree of differentiation for each neuroblastic nodule: _____

___Intermixed subtype

___Ganglioneuroma

Specify extent (%):

___Malignant peripheral nerve sheath tumor

Specify extent (%):

___Neuroblastic tumor, not otherwise specified

Specify extent (%):

___Other (specify):

†Cytologic variants of Chromaffin and/or Chief cells (select all that apply)

___Epithelioid

___Clear cell

___Spindle cell

___Lipid cell change

___Oncocytic change

Necrosis

___Not identified

___Present, focal (small microscopic foci or single cell necrosis)

___Present, extensive (central, expansive or “comedo” necrosis)

Mitotic rate (select all that apply)

††Based upon counting 50 high-power fields (HPF: 40x objective) and in the area of highest mitotic activity, and reported as number of mitoses per 10 HPF)

†††Alternatively the mitotic count can be given as per mm^2

_____Specify mitoses (per 10 HPF or mm^2): _____

_____Atypical mitoses

_____Cannot be determined

†Additional Features

___Hyaline globules

___Amyloid deposition

___Neuromelanin deposition

___Myxoid and/or hyaline stroma

___Degeneration (specify): _____

Encapsulation and Invasion (note G)

___Thick capsule

___No capsule

___Cannot be determined

Invasive growth (select all apply)

Tumor capsule invasion (transcapsular)

___Present

Specify the extent of invasion (number of foci): _____

___Not identified

___Indeterminate

___Cannot be assessed

Adrenal capsule invasion (transcapsular)

___Present

Specify the extent of invasion (number of foci): _____

___Not identified

___Indeterminate

___Cannot be assessed

Local invasion into surrounding tissues

___Present

Specify tissues: _____

Specify extent (gross or microscopic): _____

___Not identified

___Indeterminate

___Cannot be assessed

Vascular invasion (intravascular tumor cells associated with thrombus)

Intracapsular

___Present

Specify the extent of invasion (number of vessels involved): _____

___Not identified

___Indeterminate

___Cannot be assessed

Beyond capsule

___Present

Specify the extent of invasion (number of vessels involved): _____

___Not identified

___Indeterminate

___Cannot be assessed

Lymphatic invasion

___Present

___Not identified

___Indeterminate

___Cannot be assessed

Surgical margins

___Uninvolved

†Distance to closest margin: _____

___Involved

___Gross

___Microscopic

___Cannot be assessed

___Other (specify): _____

Metastases (note H)

___Lymph node metastases

___Present

___Not identified

___Indeterminate

___Number of lymph nodes examined

___Number of metastatic lymph nodes:

†Number of lymph nodes with macrometastases (>2 mm): ___

†Number of lymph nodes with micrometastases (≤0.2 mm): ___

___Extranodal extension

___Present

___†Focal (microscopic)

___†Extensive

___Not identified

___Indeterminate

___Distant metastases

___Present (specify site and data source): _____

___Not identified

___Cannot be determined

†Immunohistochemistry (Check all positive or select all that apply) (note I)

___Chromogranin A

___Synaptophysin

___Tyrosine hydroxylase

___S100 protein (sustentacular cells)

___Loss of SDHB expression

___Loss of SDHA expression

MIB-1 (Ki-67) LI (percentage of positive tumor cells in area of highest nuclear labeling): __ %

Others (specify):_____

† **Associated Lesions (note J)**

___Adrenal medullary hyperplasia

___Current or past tumors in other organs (specify): _____

†**Clinicopathologic Correlation** (Check all that apply)

___Evidence of hereditary disease

Clinical

Family history (specify): _____

Associated lesions (specify): _____

Biochemical profile (specify): _____

Cannot be assessed

Pathological

___Multiple pheochromocytoma/paraganglioma

___Adrenal medullary hyperplasia

Immunohistochemistry (specify): _____

Cannot be assessed: _____

†Comment(s):_____

EXPLANATORY NOTES

A: Anatomical Sites of Paraganglia—Paraganglia are neural crest-derived neuroendocrine organs that produce predominantly catecholamines [1-3]. Paraganglia are typically divided into two groups based on parasympathetic or sympathetic nervous system origin. Sympathetic paraganglia are also divided into two subgroups: the adrenal medulla, so-called “sympathoadrenal paraganglia” and extra-adrenal sympathetic paraganglia [3-5]. The anatomic site impacts the nomenclature of tumors arising from paraganglia; while tumors arising from the adrenal medulla are termed “pheochromocytomas”, tumors arising from extra-adrenal locations are called “paragangliomas” regardless of their sympathetic or

parasympathetic origins [1-5]. Furthermore, the anatomic site of a tumor predicts the risk of malignancy, since extra-adrenal paragangliomas exhibit a higher risk of malignancy [1-8].

B: Clinical and Biochemical Features—While pheochromocytomas and the majority of sympathetic paragangliomas are often associated with clinical symptoms, only a small percentage of parasympathetic paragangliomas are symptomatic [1]. Many clinically silent paragangliomas, particularly of the sympathoadrenal type will produce metanephrines and/or methoxytyramine and therefore be amenable to biochemical testing [7, 9]. However, parasympathetic paragangliomas often lack tyrosine hydroxylase, the enzyme required for catecholamine synthesis, and are therefore usually non-functional [2].

Similar to other neuroendocrine neoplasms, pheochromocytomas and extra-adrenal paragangliomas are also capable of producing and secreting other peptides that can cause clinical syndromes. Production of ACTH (adrenocorticotropic hormone), β-endorphin, CRH (corticotropin-releasing hormone), calcitonin gene-related peptide, VIP (vasoactive intestinal peptide), GHRH (growth hormone-releasing hormone), neuropeptide Y, peptide YY, IGF-1 (insulin like growth factor-1), galanin, adrenomedullin, serotonin, somatostatin and gastrin-like neuropeptide have been reported [1-5, 10-17].

Recent molecular data suggest genotype-phenotype correlations in paragangliomas with respect to tumor distribution, catecholamine production and risk of metastasis [2, 7, 18-23]. It is now recognized that at least 30% of paragangliomas and pheochromocytomasare associated with familial syndromes [2, 3, 18]. 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 conditions, biochemical testing for the O-methylated metabolites of dopamine, norepinephrine and epinephrine (methoxytyramine, normetanephrine and metanephrine, respectively) in plasma and/or urine is superior to measurement of the parent catecholamines [7, 22, 23]. In terms of their biochemical profile, *SDHx* (Succinate dehydrogenase, x refers to all subunits, e.g. SDHA refers to subunit A)-related tumors are associated with dopamine and/or norepinephrine production, *VHL* (Von Hippel-Lindau)-related tumors are associated with norepinephrine production, *RET* (Rearranged during transfection)- and *NF-1* (Neurofibromin 1)-related tumors are associated with epinephrine production [2, 7]. Moreover, the risk of malignancy is significantly higher in *SDHB* (Succinate dehydrogenase subunit B)-related chromaffin cell tumors, which are usually observed in extra-adrenal locations and reach larger sizes with much lower tissue concentrations of catecholamines than other paragangliomas [7, 19-23]. 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.

C: Functional Imaging: Tumor Scintigraphy or Positron Emission Tomography (PET)—Similar to the genotype-biochemical profile correlations of paragangliomas, the functional status of a paraganglioma has an impact on imaging modalities that are used to localize these lesions [18]. ^{123}I-metaiodobenzylguanidine scintigraphy (123I-MIBG) and ^{18}F-6-fluorodopamine (18F-FDA) or ^{18}F-6-fluorodihydroxphenylalanine (18F-FDOPA) PET, are superior to other functional imaging modalities for detecting pheochromocytomas [18, 24]. In contrast, ^{18}F-fluorodeoxyglucose (18-F-FDG) PET is more useful than other modalities for diagnostic localization of *SDHB*-driven metastatic paragangliomas [18, 24], whereas 18F-DOPA PET has been reported to be the most effective functional imaging modality for localization of *SDHx*-related head and neck paragangliomas [25]. Recently, it has been shown that 18F-FDOPA PET is most useful for the detection of head and neck paragangliomas and neuroendocrine neoplasms arising in

patients with VHL syndrome [26, 27]. When available, the integration of functional imaging data is of clinical interest and will ascertain the completeness of the synoptic report.

D: Tumor Location, Size, Weight and Focality—The significance of tumor location with respect to the parasympathetic/sympathetic origin of the tumor, and correlation with the biochemical profile and the appropriate terminologies are discussed in detail in parts A and B. Therefore, the anatomic location of the tumor must be clearly specified 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. Numerous reports have indicated that malignant tumors are heavier and larger than tumors with benign behavior [3, 6, 23, 28-31]. Although the tumor size and weight are not universally considered independent parameters, a cut-off of 5-6 cm diameter and 80-150 gram weight have been suggested to predict malignant behavior [23, 28-31].

The issue of multifocality is of interest and should be included in the synoptic report[1-5]. Patients with multiple paragangliomas should be investigated for the possibility of underlying genetic susceptibility and thus genetic testing for *RET*, *NF-1*, *VHL*, *SHDx*, *TMEM127* (Transmembrane protein 127), *MAX* (MYC associated factor-X) and *KIF1Bβ* (Kinesin family member 1B) mutations should be considered [1, 3, 19, 20, 32-36]. While the value of systematic genetic screening for “sporadic” cases remains controversial, clinical features including family history, along with the biochemical and morphological features (multifocality, adrenal medullary hyperplasia, thick capsule, clear cell morphology), and immunoprofile (loss of SDHB and SDHA expression) [36, 37] (see parts B, F, I and J) can provide important insight to determine which gene(s) should be screened preferentially in patients with pheochromocytomasand/or extra-adrenal paragangliomas. Multifocality includes multiple pheochromocytomas in the same adrenal.

E: Classification—Anatomic location impacts the terminology used for these tumors. In the presence of metastatic disease, the term “metastatic” should be used. The term “composite” is used when a tumor combines features of paraganglioma or pheochromocytoma with those of malignant peripheral nerve sheath tumor, ganglioneuroma, ganglioneuroblastoma and neuroblastoma. Comprehensive data related to neuroblastic and related components should be reported using the designated synoptic checklist [38]. The histological classification generated from the recommendations of the 2004 World Health Organization Classification of Tumors of Endocrine Organs [1] is listed below, however for simplicity; the format proposed is shortened to allow a practical approach for synoptic reporting.

Classification of Pheochromocytomas and Extra-Adrenal Paragangliomas

Adrenal gland

___Pheochromocytoma

___Metastatic pheochromocytoma

___Composite pheochromocytoma (specify components): _____

Extra-adrenal localizations

___Carotid body paraganglioma

___Jugulotympanic paraganglioma

___Vagal paraganglioma

___Laryngeal paraganglioma

___Aortico-pulmonary paraganglioma

___Gangliocytic paraganglioma

___Cauda equina paraganglioma

___Orbital paraganglioma

___Nasopharyngeal paraganglioma

___Extra-adrenal sympathetic paraganglioma

___Superior and inferior paraaortic paraganglioma

___Urinary bladder paraganglioma

___Intrathoracic and cervical paravertebral paraganglioma

___Metastatic paraganglioma

___Composite paraganglioma (specify site and components): _____

___Others (specify): _____

F: Histologic Features—Regardless of sympathetic or parasympathetic origin, paragangliomas usually exhibit overlapping morphologic features. They display a variety of growth patterns and cytological features[1-5]. While sympathetic paragangliomas and pheochromocytomas consist of polygonal cells, so-called “chromaffin” cells that exhibit amphophilic to basophilic cytoplasm, parasympathetic tumors consist of polygonal cells, so-called “chief cells” that often have relatively clearer cytoplasm than their sympathetic counterparts. However, overlapping of these cells is often seen in these tumors. Similar to other endocrine lesions, oncocytic change, spindle cell morphology and lipid cell degeneration leading a clear cell morphology that mimics cortical lesions can also be seen in these neoplasms [1, 3, 39].

Genotype-phenotype correlations highlighted that VHL-related tumors contain usually a thick vascular capsule, hyalinized and myxoid stroma, round tumor cells intermingled with small vessels, cells with predominantly amphophilic and clear cell cytoplasm, absence of intracytoplasmic hyaline globules, lipid degeneration [39] and lack of nuclear atypia or mitoses [1, 3].

The term “composite” should be used when a tumor combines features of paraganglioma or pheochromocytoma with those of malignant peripheral nerve sheath tumor, ganglioneuroma, ganglioneuroblastoma and neuroblastoma. Comprehensive data related to neuroblastic and related component should be reported by using the designated synoptic checklist [38]. In this setting, corticomedullary tumors, cauda equina Paragangliomasshowing ependymal differentiation as well as gangliocytic paragangliomas that include Schwann-like cells and ganglion cells do not qualify as composite tumors [1, 4]. Moreover, scattered mature ganglion cells seen in pheochromocytomas/paragangliomas should not be misinterpreted as a component of a composite tumor [1-4].

No single histological parameter is able to predict malignant behavior in paragangliomas and pheochromocytomas [1-5]. Tumor necrosis is uncommon in these tumors and degenerative changes should not be mistaken as necrosis [1-5]. However, expanded large confluent nests with central comedo necrosis, which are at least three times greater than conventional small nests, have been described in some malignant pheochromocytomas/paragangliomas [1-4]. Therefore, a distinction should be made between focal (small microscopic foci or single cell necrosis) and extensive (central, expansive or "comedo" necrosis). Increased mitoses (>3/10 High Power Fields, HPF) and atypical mitotic figures have been reported in some malignant cases [1, 3, 4], but mitoses are usually very rare even in malignant cases. There is currently no standard approach to mitotic count in pheochromocytoma/paraganglioma. On the basis of established methodology for other neuroendocrine tumors, it has been recommended that mitotic count should be based upon counting 50 HPF (40x objective) and in the area of highest mitotic activity, and reported as number of mitoses per 10 HPF. However, taking into consideration the variations in field size, providing the number of mitosis per mm^2 seems to be more appropriate. The College of American Pathologist Breast Cancer Protocol[40] recommends that the size of HPF be measured by using a micrometer. Alternatively, it has been suggested that that the high power field diameter/area can also be calculated by using the following formulas[40]: (a) Measure the diameter of a low-power field by using a ruler, (b) Calculate a constant by using the following formula: Eyepiece Magnification x Objective Magnification x Microscopic Field Diameter= A Constant, (c) Calculate the diameter of an HPF for other objectives by using the following formula: Unknown Field Diameter = Constant/(Eyepiece Magnification x Objective Magnification), (d) Calculate the area of the HPF as follows (Half of the field diameter is the radius of the field (r)); $3.1415 \times r^2$ = Area of Microscopic Field. By doing this, one can also provide the mitotic activity per mm^2.

G: Encapsulation and Invasiveness—According to the 2004 World Health Organization (WHO) classification of endocrine neoplasms, malignancy of pheochromocytomas and extra-adrenal paragangliomas is defined by the presence of metastases to sites where paraganglial tissue is not normally found[1]. Although local gross invasion into the adjacent organs is considered in the definition of malignancy proposed by the 2007 Armed Forces Institute of Pathology (AFIP) fascicle [4], this is not regarded as a strong predictor of metastases and therefore it is not integrated in the 2004 WHO classification [1-3]. Moreover, unlike other neoplasms, vascular invasion is also not universally accepted as an unequivocal predictor of malignant potential in paragangliomas and pheochromocytomas [1-3]. However, it is important to document the invasiveness of these tumors. Moreover, strict criteria to diagnose vascular invasion (intravascular tumor cells associated with thrombus) and capsular invasion (transcapsular) should be applied as they are in other endocrine organs [41]. As discussed in part F, the presence of a thick vascular capsule may raise the suspicion of a VHL-related paraganglioma [1-3].

H: Metastases—An extra-adrenal location, large size, and the presence of *SDHB* mutations are all important risk factors for metastatic spread [6, 7, 21, 23, 42]. High rates of malignancy in tumors associated with *SDHB* mutations can be fully accounted by both their typically extra-adrenal location and large size [23]. While tumors arising from head and neck paragangliomas are much less often metastatic, mediastinal and intraabdominal paragangliomas appear to often be associated with metastatic disease [1, 3, 6, 8].

The diagnosis of metastasis is appropriate when dealing with a site where no paraganglial tissue is observed; it is crucial to remember the normal anatomic distribution of paraganglia as discussed in part A, to consider the possibility of multifocal primaries. The pathology report should state the total number of lymph nodes examined, the number of nodes with metastases, and nodal involvement should be reported as macrometastasis (>2mm) or

micrometastasis (≤2mm and including isolated tumor cells) based on the size of the metastatic deposit. While the determination of the nodal disease is easy, the assessment of distant metastasis can be challenging in the setting of multifocal disease, since primary paragangliomasdo also occur in rare anatomical sites such as thyroid, pituitary, gallbladder, and lung [2, 4, 43-45]. Therefore, these rare locations should not be considered metastatic ab initio.

I: Immunohistochemistry—Positivity for tyrosine hydroxylase, which is the rate limiting enzyme in the synthesis of catecholamines [46], is very helpful to distinguish paragangliomas from other neuroendocrine carcinomas, which can also be negative for cytokeratins [2]. However, positivity for chromogranin-A and tyrosine hydroxylase is usually weaker and more variable in parasympathetic paragangliomas than in sympathetic paragangliomas and is sometimes negative[2]. Some of these tumors selectively express chromogranin B.

S100 protein is typically used to highlight the sustentacular network in paragangliomas; however, the reactivity pattern is usually variable. It is of note that epithelioid endocrine cells and spindled Schwann-like cells of gangliocytic paragangliomas can be positive for cytokeratin and S100 protein, respectively [1, 4]. Moreover, cauda equina paragangliomas, which are usually intradural lesions limited to the filum terminale, may show ependymal and neuronal differentiation and can be positive for cytokeratin [1, 4, 47].

There is currently no standard approach to scoring Ki-67 in pheochromocytomaand paraganglioma. On the basis of established methodology for other neuroendocrine tumors, it is recommended that Ki-67 index should be reported as percentage of positive tumor cells in area of highest nuclear labeling [48].

Loss of SDHB expression is regarded as a surrogate marker for some of the familial paraganglioma syndromes caused by *SDHx* mutations [36], therefore immunohistochemical testing for SDHB has become a part of the routine assessment of these lesions in many centers. Moreover, the use of SDHB antibody not only allows the identification of *SDHx* related tumors, but also provides prognostic data, due to the high rate of malignancy associated with SDHB-driven paragangliomas [7, 21, 24, 42]. Recently, it was also demonstrated that SDHA immunohistochemistry is also very useful to reveal the presence of *SDHA* germline mutations [37]; PGLs associated with germline *SDHA* mutation show negative staining for SDHA as well as SDHB.

J: Associated Lesions—It is widely accepted that adrenal medullary hyperplasia is a precursor lesion of pheochromocytomas arising in MEN 2 (Multiple endocrine neoplasia type 2) syndromes and is characterized by a nodular and/or diffuse enlargement of the adrenal medulla [1-5]. Although other predisposing genetic syndromes are not usually associated with adrenal medullary hyperplasia, it is noteworthy that a 61-year-old man with an SDHB mutation was found to have bilateral adrenal medullary hyperplasia characterized by an increased cortex to medulla ratio of 1:1 in both glands [49]. Other exceptions might also exist.

The determination of underlying adrenal medullary hyperplasia is one of the clinical responsibilities of pathologists examining adrenal glands. When examining diffuse hyperplasia, it is important to remember that medulla is normally present only in the head and body, but not in the tail of the gland with only minimal extension into the alae [3-5]. Although it is sometimes hard to define the tail of the adrenal due to distortion of the gland by tumor, the presence of adrenal medullary tissue in the tail qualifies as adrenal medullary hyperplasia [3-5]. In general, medulla should not represent more than one-third of the gland

thickness, with cortex on each side comprising the other two thirds. The distinction of pheochromocytoma from nodular adrenal medullary hyperplasia is arbitrary since even microscopic nodules observed in the setting of MEN 2 syndrome represent clonal proliferations; therefore they are indeed neoplastic lesions [3, 5]. However, nodules less than 1 cm can be practically considered to represent hyperplasia [4], provided that they grossly and microscopically resemble the rest of the medulla. It should be remembered that adrenal medullary nodules and pheochromocytomas can occur in MEN 2 adrenals without an obvious background of diffuse hyperplasia. The adrenal adjacent to an apparently sporadic pheochromocytoma should therefore be “breadloafed” and carefully examined for small nodules.

References

1. DeLellis, RA.; Lloyd, RV.; Heitz, PU.; Eng, C. Pathology and Genetics of Tumours of Endocrine Organs. IARC Press; Lyon, France: 2004.
2. Tischler AS. Pheochromocytoma and extra-adrenal paraganglioma: updates. Arch Pathol Lab Med. 2008; 132(8):1272–1284. [PubMed: 18684026]
3. McNicol, AM. Adrenal medulla and paraganglia.. In: Llyod, RV., editor. Endocrine Pathology: Differential Diagnosis and Molecular Advances. Springer; 2010. p. 281-295.
4. Lack, EE. Tumors of the adrenal glands and extraadrenal paraganglia. ARP Press; Washington, DC: 2007. AFIP atlas of tumor pathology, fourth series, fascicle 8..
5. Asa, SL.; Fischer, SE. Adrenal gland.. In: Gattuso, P.; Reddy, VB.; David, O.; Spitz, DJ.; Haber, MH., editors. Differential Diagnosis in Surgical Pathology. Saunders Elsevier; 2010. p. 461-485.
6. Korevaar TIM, Grossman AB. Pheochromocytomas and paragangliomas: assessment of malignant potential. Endocrine. 2011; 40(3):354–365. [PubMed: 22038451]
7. Eisenhofer G, Tischler AS, de Krijger RR. Diagnostic tests and biomarkers for pheochromocytoma and extra-adrenal paraganglioma: From routine laboratory methods to disease stratification. Endocr Pathol. 2012; 23(1):4–14. [PubMed: 22180288]
8. Ayala-Ramirez M, Feng L, Johnson MM, et al. Clinical risk factors for malignancy and overall survival in patients with pheochromocytomas and sympathetic paragangliomas: primary tumor size and primary tumor location as prognostic indicators. J Clin Endocrinol Metab. 2011; 96(3):717–725. [PubMed: 21190975]
9. Eisenhofer G, Siegert G, Kotzerke J, Bornstein SR, Pacak K. Current progress and future challenges in the biochemical diagnosis and treatment of pheochromocytomas and paragangliomas. Horm Metab Res. 2008; 40(5):329–337. [PubMed: 18491252]
10. Schroeder JO, Asa SL, Kovacs K, Killinger D, Hadley GL, Volpé R. Report of a case of pheochromocytoma producing immunoreactive ACTH and beta-endorphin. J Endocrinol Invest. 1984; 7(2):117–121. [PubMed: 6327797]
11. Bayraktar F, Kebapcilar L, Kocdor MA, et al. Cushing's syndrome due to ectopic CRH secretion by adrenal pheochromocytoma accompanied by renal infarction. Exp Clin Endocrinol Diabetes. 2006; 114(8):444–447. [PubMed: 17039427]
12. Herrera MF, Stone E, Deitel M, Asa SL. Pheochromocytoma producing multiple vasoactive peptides. Arch Surg. 1992; 127(1):105–108. [PubMed: 1734841]
13. Asa SL, Kovacs K, Thorner MO, Leong DA, Rivier J, Vale W. Immunohistological localization of growth hormone-releasing hormone in human tumors. J Clin Endocrinol Metab. 60(3):423–427. 198. [PubMed: 3919041]
14. Grouzmann E, Comoy E, Bohuon C. Plasma neuropeptide Y concentrations in patients with neuroendocrine tumors. J Clin Endocrinol Metab. 1989; 68(4):808–813. [PubMed: 2466046]
15. Bauer FE, Hacker GW, Terenghi G, Adrian TE, Polak JM, Bloom SR. Localization and molecular forms of galanin in human adrenals: elevated levels in pheochromocytomas. J Clin Endocrinol Metab. 1986; 63(6):1372–1378. [PubMed: 2430990]
16. Thouennon E, Pierre A, Yon L, Anouar Y. Expression of trophic peptides and their receptors in chromaffin cells and pheochromocytoma. Cell Mol Neurobiol. 2010; 30(8):1383–1389. [PubMed: 21046451]

17. Warren WH, Lee I, Gould VE, Memoli VA, Jao W. Paragangliomas of the head and neck: ultrastructural and immunohistochemical analysis. Ultrastruct Pathol. 1985; 8(4):333–343. [PubMed: 3001985]

18. Gimenez-Roqueplo AP, Tischler AS. Pheochromocytoma and Paraganglioma: Progress on all fronts. Endocr Pathol. 2012; 23(1):1–3. [PubMed: 22246920]

19. Cascon A, Tennant DA. From transcriptional profiling to tumor biology in pheochromocytoma and paraganglioma. Endocr Pathol. 2012; 23(1):15–20. [PubMed: 22323008]

20. Nolthing S, Grossman AB. Signaling Pathways in Pheochromocytomas and Paranganglíomas: Prospects for future therapies. Endocr Pathol. 2012; 23(1):21–33. [PubMed: 22391976]

21. Gimenez-Roqueplo AP, Favier J, Rustin P, et al. Mutations in the SDHB gene are associated with extra-adrenal and/or malignant phaeochromocytomas. Cancer Res. 2003; 63(17):5615–5621. [PubMed: 14500403]

22. Eisenhofer G. Screening for pheochromocytomas and paragangliomas. Curr Hypertens Rep. 2012; 14(2):130–137. [PubMed: 22258313]

23. Eisenhofer G, Lenders JW, Siegert G, et al. Plasma methoxytyramine: A novel biomarker of metastatic pheochromocytoma and paraganglioma in relation to established risk factors of tumour size, location and SDHB mutation status. Eur J Cancer. 2012; 48(11):1739–1749. [PubMed: 22036874]

24. Chen H, Sippel RS, O'Dorisio MS, et al. The North American Neuroendocrine Tumor Society consensus guideline for the diagnosis and management of neuroendocrine tumors: pheochromocytoma, paraganglioma, and medullary thyroid cancer. Pancreas. 2010; 39(6):775–783. [PubMed: 20664475]

25. King KS, Chen CC, Alexopoulos DK, et al. Functional imaging of SDHx-related head and neck paragangliomas: comparison of 18F-fluorodihydroxyphenylalanine, 18F-fluorodopamine, 18F-fluoro-2-deoxy-D-glucose PET, 123I-metaiodobenzylguanidine scintigraphy, and 111In-pentetreotide scintigraphy. J Clin Endocrinol Metab. 2011; 96(9):2779–2785. [PubMed: 21752889]

26. Weisbrod AB, Kitano M, Gesuwan K, et al. Clinical Utility of Functional Imaging with 18F-FDOPA in Von Hippel-Lindau Syndrome. J Clin Endocrinol Metab. 2012; 97(4):E613–617. [PubMed: 22259055]

27. Zelinka T, Musil Z, Duskova J, et al. Metastatic pheochromocytoma: Does the size and age matter? Eur J Clin Invest. 2011; 41(10):1121–1128. [PubMed: 21692797]

28. Feng F, Zhu Y, Wang X, et al. Predictive factors for malignant pheochromocytoma: analysis of 136 patients. J Urol. 2011; 185(5):1583–1590. [PubMed: 21419457]

29. Linnoila RI, Keiser HR, Steinberg SM, Lack EE. Histopathology of benign versus malignant sympathoadrenal paragangliomas: clinicopathologic study of 120 cases including unusual histologic features. Hum. Pathol. 1990; 21(11):1168–1180. [PubMed: 2172151]

30. John H, Ziegler WH, Hauri D, Jaeger P. Pheochromocytomas: Can malignant potential be predicted? Urology. 1999; 53(4):679–683. [PubMed: 10197840]

31. Burnichon N, Cascón A, Schiavi F, et al. MAX Mutations Cause Hereditary and Sporadic Pheochromocytoma and Paraganglioma. Clin Cancer Res. 2012; 18(10):2828–2837. [PubMed: 22452945]

32. Qin Y, Yao L, King EE, et al. Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. Nat Genet. 2010; 42(3):229–233. [PubMed: 20154675]

33. Buffet A, Venisse A, Nau V, et al. A Decade (2001-2010) of Genetic Testing for Pheochromocytoma and Paraganglioma. Horm Metab Res. 2012; 44(5):359–366. [PubMed: 22517557]

34. Yeh IT, Lenci RE, Qin Y, et al. A germline mutation of the KIF1B beta gene on 1p36 in a family with neural and nonneural tumors. Hum Genet. 2008; 124(3):279–285. [PubMed: 18726616]

35. Chetty R. Familial paraganglioma syndromes. J Clin Pathol. 2010; 63(6):488–491. [PubMed: 20498024]

36. van Nederveen FH, Gaal J, Favier J, et al. An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene

mutations: a retrospective and prospective analysis. Lancet Oncol. 2009; 10(8):764–771. [PubMed: 19576851]

37. Korpershoek E, Favier J, Gaal J, et al. SDHA immunohistochemistry detects germline SDHA gene mutations in apparently sporadic paragangliomas and pheochromocytomas. J Clin Endocrinol Metab. 2011; 96(9):E1472–1476. [PubMed: 21752896]
38. Jarzenmbowski, JA.; Bowen, J.; Hill, DA.; Shimada, H. College of American Pathologists, Protocol for the examination of specimens from patients with neuroblastoma. Jun. 2012 available at: http://www.cap.org/apps/cap.portal
39. Ramsay JA, Asa SL, van Nostrand AW, Hassaram ST, de Harven EP. Lipid degeneration in pheochromocytomas mimicking adrenal cortical tumors. Am J Surg Pathol. 1987; 11(6):480–486. [PubMed: 3592062]
40. Lester, SC.; Bose, S.; Chen, YY., et al. College of American Pathologists, Protocol for the examination of specimens from patients with neuroblastoma. Apr. 2013 available at: http://www.cap.org/apps/cap.portal
41. Mete O, Asa SL. Pathological definition and clinical significance of vascular invasion in thyroid carcinomas of follicular epithelial derivation. Mod Pathol. 2011; 24(12):1545–1552. [PubMed: 21804527]
42. Brouwers FM, Eisenhofer G, Tao JJ, et al. High frequency of SDHB germline mutations in patients with malignant catecholamine-producing paragangliomas: implications for genetic testing. J Clin Endocrinol Metab. 2006; 91(11):4505–4509. [PubMed: 16912137]
43. Phitayakorn R, Faquin W, Wei N, Barbesino G, Stephen AE. Thyroid-associated paragangliomas. Thyroid. 2011; 21(7):725–733. [PubMed: 21615305]
44. Sambaziotis D, Kontogeorgos G, Kovacs K, Horvath E, Levedis A. Intrasellar paraganglioma presenting as nonfunctioning pituitary adenoma. Arch Pathol Lab Med. 1999; 123(5):429–432. [PubMed: 10235503]
45. Mehra S, Chung-Park M. Gallbladder paraganglioma: a case report with review of the literature. Arch Pathol Lab Med. 2005; 129(4):523–526. [PubMed: 15794679]
46. Kimura N, Miura Y, Nagatsu I, Nagura H. Catecholamine synthesizing enzymes in 70 cases of functioning and non-functioning phaeochromocytoma and extraadrenal paraganglioma. Virchows Arch A Pathol Anat Histopathol. 1992; 421(1):25–32. [PubMed: 1353277]
47. Matsumoto M, Abe K, Baba H, et al. Paraganglioma of the cauda equina: a report of two cases with unusual histopathological features. Clin Neuropathol. 2012; 31(1):39–43. [PubMed: 22192703]
48. Adsay V. Ki67 labeling index in neuroendocrine tumors of the gastrointestinal and pancreatobiliary tract: to count or not to count is not the question, but rather how to count. Am J Surg Pathol. 2012; 36(12):1743–1746. [PubMed: 23154766]
49. Grogan RH, Pacak K, Pasche L, Huynh TT, Greco RS. Bilateral adrenal medullary hyperplasia associated with an SDHB mutation. J Clin Oncol. 2011; 29(8):e200–202. [PubMed: 21172883]