Recent Developments in the Diagnosis of Adrenal Neoplasia

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- Distinguishing adrenal cortical adenomas from carcinoma relies on the Weiss criteria which are a very useful guideline.
- Criteria for malignancy of adrenal cortical carcinomas in pediatric patients are different from those in adults.
- In addition to typical adrenal cortical tumors, oncocytic and myxoid variants may also be present.
- There are several new systems to evaluate malignancy in pheochromocytomas. However, the presence of metastatic disease remains the most reliable predictor of malignancy.
Adrenal Cortical Tumors

One of the major problems in diagnostic endocrine pathology is distinguishing adrenal cortical adenomas from carcinomas. This is especially true with borderline lesions compared to small adenomas less than 20 grams or very large tumors more than 500 grams that are usually obvious carcinomas.

Adrenal cortical adenomas may be functioning or non-functioning (1). Many small adenomas less than 3 cm in diameter are discovered accidentally during working up for various other conditions. These tumors are referred to as “incidentalomas”.

Adrenal cortical carcinoma may be functioning malignancies in some cases, while in other cases, they are non-functioning (1-6). The gross appearance of adrenal cortical carcinoma can be very helpful in making the diagnosis. Most carcinomas in adults are greater than 100 grams, while adenomas generally weigh 50 grams or less. Adrenal cortical tumors weighing less than 50 grams have, on occasion, metastasized, but this is extremely uncommon. In pediatric patients, however, adrenal cortical adenomas may weigh up to 500 grams. In addition to tumor weight, the presence of necrosis usually indicates an adrenal cortical carcinoma unless the necrosis resulted from a traumatic insult such as FNA. A variegated appearance with nodularity and intersecting fibrous bands should also suggest the possibility of a carcinoma.

Various studies have outlined specific criteria used to diagnose adrenal cortical carcinomas (7-10). The criteria of Weiss (8) are most useful because of their reliance on histologic features. These include high nuclear grade, mitotic rate greater than 5 per 50 high power fields, atypical mitotic figures, eosinophilic tumor cells (≥ 75%), diffuse architecture (> 33% of tumor), necrosis, venous invasion, sinusoidal invasion and capsular invasion (8).
Three of more of the nine criteria are indicative of an adrenal cortical carcinoma, while two or less would be more in keeping with an adenoma. Other systems such as that of van Slooten et al., (11) attach numeric values to the various criteria, and an index of eight or higher was consistent with a carcinoma.

One of the older systems is that of Hough et al (12) who used histologic criteria and clinical parameters in their assessment of adrenal cortical neoplasms. A numeric value of 2.91 was indicative of malignancy, while a value of 0.17 or less was consistent with a benign lesion. The disadvantage of this system is the reliance on clinical parameters as well as histologic features, and some of these clinical parameters may not be available when examining the specimen.

From a practical perspective, the most useful criteria to separate adenomas from carcinomas include tumor size, presence of necrosis, mitotic activity including atypical mitoses, invasive growth, and high nuclear grade. Capsular invasion may be difficult to recognize because the expanding capsule may be a pre-existing adrenal capsule. Invasion of adjacent soft tissue, kidney or liver, are definitive signs of malignancy. Special studies may be useful in confirming the nature of the malignant tissue (12-19). Ultrastructural studies may show the distinct features of ACC tissues including abundant smooth endoplasmic reticulum and mitochondria with prominent tubular or vesicular cristae. Immunohistochemical studies that are most useful in adrenal cortical carcinoma include melan A, inhibin alpha and calretinin. Stains for cytokeratin are usually weakly positive, while vimentin is strongly positive. Synaptophysin is usually weakly positive in these tumors. Chromogranin is consistently negative. A marker for adrenal cortical cells, Ad4BP/SP-1, is relatively restricted in its distribution (20,21) and may be
useful in the diagnosis of adrenal cortical tissues. This protein is a transcription factor that is needed for embryonic development of adrenal cortical cells.

Adrenal cortical neoplasm in pediatric patients is more difficult to diagnose and to separate adenomas from carcinomas (22). In a study of 83 adrenal cortical neoplasms, only 31% of histologically malignant tumors behaved in a clinically malignant fashion. Features of malignancy included tumor weight >400 g, tumor size >10.5 cm, vena cava invasion, confluent necrosis, periadrenal soft tissue invasion greater than 15 mitoses per hpf and atypical mitoses.

There are several variants of adrenal cortical tumors. The most common include the oncocytic tumors (23,24), and the myxoid variant (25). Criteria for the diagnosis of oncocytic carcinomas were recently proposed by Weiss’ group (24). Major criteria for oncocytic tumors included high mitotic rate, atypical mitoses and vena cava invasion. Minor criteria included large size and weight, necrosis, capsular invasion and sinusoidal invasion. One major criteria indicated malignancy, while 1 to 4 minor criteria indicated borderline tumors. Absence of all major and minor criteria indicated benign oncocytic tumors. The myxoid variant of adrenal cortical tumors looks different morphologically, but the criteria for malignancy should be similar to conventional adrenal cortical tumors.

The differential diagnosis of adrenal cortical carcinoma includes renal cell carcinoma, hepatocellular carcinoma, pheochromocytomas, and metastatic carcinomas and melanomas. Insulin-like growth factor-2 has been useful in the classification of adrenal cortical tumors (26).

Recent studies of these various markers and techniques separate adrenal cortical carcinomas from adenomas. Some of these include DNA flow cytometric analysis and nucleolar organizer regions have not been very effective. However, some markers of proliferation have
been shown to be useful in the distinction. Ki-67 labeling index with MIB-1 antibody are somewhat promising (19,20).

**Molecular Studies**

Molecular studies have characterized various genes that are differentially expressed in normal and benign compared to malignant adrenal cortical tumors (27). The phenotypes of Ki-67 negative, p53 negative, mdm-2 positive, cyclin D1 negative, Bcl-2 negative, p21 negative and p27 positive cells was found in 83% of normal adrenal tissues, but only in 3% of malignant tumors (27). Giordano and colleagues performed microarray analysis of adrenal cortical tumors and reported up-regulation of IGF2 in 10% of adrenal cortical carcinomas (90.9%). Proliferation in related genes such as TOP2A and Ki-67 were also up-regulated in carcinomas (28,29). Velazquez-Fernandez, et al (30) performed expression profiling of 7 patients with adrenal cortical carcinomas and 13 with adenomas and reported up-regulation of ubiquitin-related genes (USP4 and UFD1L) and insulin-like growth factor related genes (IGF2, IGF2R, IGFβP3 and IGFβP6). A cytokine gene (CXCL10) and cadherin 2 gene (CDH2) were down-regulated in carcinomas compared to adenomas (30).

**Pheochromocytomas**

Pheochromocytomas (“dusky colored tumor”) are chromaffin derived tumors that develop in the adrenal gland (31). When the tumor is immersed in chromaffin salts or other weak oxidizing agents, it develops the dusky color. Most tumors are sporadic and benign. The reported incidence is about 0.4 to 9.5 per 10^6 people. The tumors occur most frequently in the 4th
and 5th decades. Familial tumors develop at a younger age. Most familial tumors are bilateral, while sporadic tumors are unilateral.

Patients usually present with throbbing headaches, sweating, palpitations, chest and abdominal pains. The “spells” may last from 10 to 60 minutes and may be triggered by positional changes.

Malignant pheochromocytomas comprise only about 10% of all pheochromocytomas. Signs and symptoms are similar to those in patients with benign disease; however, catecholamine production and the degree of hypertension may be more marked with metastatic disease.

Imaging studies cannot distinguish benign from malignant pheochromocytomas unless there is metastatic disease. CT studies and 123I-meta-iodobenzyl-guanidine (MIBG) is very useful in imaging especially for locally recurrent or metastatic disease.

Malignant pheochromocytomas tend to be larger tumors than benign ones. They may be more nodular, lobular and show areas of necrosis. They may infiltrate periairrenal adipose tissue. Metastatic disease is the most reliable evidence of malignancy (32-45).

Histological features suggesting malignancy include: capsular invasion, vascular invasion, extension into periairrenal adipose tissue, diffuse growth, necrosis, tumor cell spindling, increased cellularity, marked nuclear pleomorphism. Macronucleoli, increased mitoses including atypical mitoses, absence or decreased hyaline globules.

Sustentacular cells were reported to be decreased or absent in malignant pheochromocytomas (31). MIB 1 labeling index may be helpful in separating benign and malignant pheochromocytomas. However, in some larger studies using 2.5% or 3.0% of cut off points had a sensitivity of only 50% in identifying proven malignant tumors.
The Pheochromocytomas of the Adrenal Gland Scaled Score (PASS) was developed by Thompson to distinguish benign from malignant pheochromocytomas (46). It uses features such as growth pattern, necrosis, cellularity, cellular monotony, tumor cell spindling, mitotic count, atypical mitosis, invasion, nuclear pleomorphism and hyperchromasia to try to separate tumors. A PASS score of ≥4 is associated with a higher probability for malignancy. Other studies of malignant pheochromocytomas have been recently reported (47-50). The proposed system of Kimura et al. used an assigned score that adds up to a maximum of 10 (50) Ki-67 immunoreactivity along with catecholamine and phenotype are included. With a score of 7 to 10, 100% of patients were found to have malignant tumors (50).

Paragangliomas are tumors arising from the paraganglia which are distributed along the parasympathetic nerves in the head, neck and mediastinum and along the sympathetic chain such as the cervical, intrathoracic, supraneural inferior paraaortic and urinary bladder. Although morphologic distinction between pheochromocytomas and paragangliomas is difficult, molecular differences between tumors arising in the adrenal medulla and other sites are more evident. With respect to malignancy, the general impression is that tumors arising in the organs of Zuckerkandl close to the bifurcation of the aorta have the highest incidence of malignancy.

Histopathologic features of pheochromocytomas and paragangliomas include chief cells with basophilic to amphophilic cells with abundant cytoplasm and large vesicular nuclei. A prominent Zellballen or cell nesting pattern may be present. Some tumors may have scant cytoplasm. Cellular and nuclear pleomorphism may be prominent. Cytoplasmic hyaline globules are frequently present. Melanin-like pigment may be present. Mitotic figures are uncommon. Tumors may have scattered ganglion cells which does not indicate a composite tumor.
Immunohistochemical studies show that the chief cells of the tumors are positive for chromogranin and synaptophysin. The sustentacular cells are positive for S100 acidic protein. The absence of positivity for EMA helps to distinguish pheochromocytomas from renal cell carcinomas. Adrenal cortical tumors are positive for melan A, inhibin alpha and calretinin and weakly positive for keratin; but negative for chromogranin A. Pheochromocytomas and paragangliomas are positive for chromogranin A and negative for melan A and keratins.

Molecular Genetics

Pheochromocytomas associated with a variety of inherited conditions including MEN2, Von Hippel-Lindau (VHL) disease, neurofibromatous type 1 (NF1), heredity paraganglioma (PGL) syndromes and Sturge-Weber disease (Table 1). The genetics of these disorders are summarized in a recent report(51).

Table 1 Hereditary conditions associated with pheochromocytomas and paragangliomas

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Chromosome Location</th>
<th>Pheo</th>
<th>PGL</th>
<th>Genetics</th>
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<tbody>
<tr>
<td>MEN 2A &amp; 2B</td>
<td>10q11.2</td>
<td>+</td>
<td></td>
<td>RET mutation</td>
</tr>
<tr>
<td>Von Hippel Lindau</td>
<td>3p26-29</td>
<td>+</td>
<td></td>
<td>VHL mutation</td>
</tr>
<tr>
<td>Neurofibromatosis I</td>
<td>17q11.2</td>
<td>+</td>
<td></td>
<td>NF1 mutation</td>
</tr>
<tr>
<td>Familial PGL1</td>
<td>11q23</td>
<td></td>
<td>+</td>
<td>SDHD mutation</td>
</tr>
<tr>
<td>Familial PGL3</td>
<td>1q2</td>
<td></td>
<td>+</td>
<td>SDHC mutation</td>
</tr>
<tr>
<td>Familial PGL4</td>
<td>1p36</td>
<td>+</td>
<td>+</td>
<td>SDHB mutation</td>
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</table>
Multiple Endocrine Neoplasia Type 2 (MEN2):

Approximately 50% of patients with MEN2 develop pheochromocytomas. De novo germ line mutations occur in about 6% of MEN2A and familial medullary thyroid carcinoma (FMTC) cases and in around 50% of MEN2B cases.

Von Hippel-Lindau (VHL):

The frequency of pheochromocytomas in VHL patients ranges from 10-30% and is restricted to the type 2 kindreds. Type 1 VHL patients with renal cell carcinomas, hemangioblastomas and retinal angiomas do not usually develop pheochromocytomas.

Neurofibromatosis Type 1 (NF):

Pheochromocytomas are associated with 1-4% of NF1 patients.

Hereditary Paraganglioma Syndromes (PGL):

The frequency of SDHB and SDHD mutations in pheochromocytomas is about 3 to 5%. These mutations are much more common in paragangliomas or extra-adrenal pheochromocytomas. SDHB mutations have been associated with malignant paragangliomas. The various PGL1 mutations are shown in Tables 1 and 2.
Table 2 Features of paragangliomas/pheochromocytomas with SDH mutations.

<table>
<thead>
<tr>
<th>Mutations</th>
<th>PGL</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDHD</td>
<td>PGL1</td>
<td>Multifocal, extra-adrenal disease &lt;age 35. If mutation will develop paragangliomas or pheochromocytomas.</td>
</tr>
<tr>
<td>SDHB</td>
<td>PGL4</td>
<td>More common in sympathetic paragangliomas associated with malignancy.</td>
</tr>
<tr>
<td>SDHC</td>
<td>PGL3</td>
<td>More common in head and neck paragangliomas. Usually benign. Rare in adrenal.</td>
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25. Brown FM, Faggey TA, Wold LE, Lloyd RV: Myxoid neoplasms of the adrenal


