Diagnostic Dilemmas in Adrenal Hyperplasia / Adenoma / Carcinoma

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Introduction

The adrenal gland comprises the cortex, responsible for the production of steroid hormones, and the medulla, producing the catecholamines norepinephrine and epinephrine. Both of these components can show hyperplastic and neoplastic change. This presentation will outline relevant aspects of normal structure and function. Then, with respect to the adrenal cortex, the various types of hyperplasia will be discussed, highlighting the differences between the types. The presentation of neoplasia will concentrate on the difficulties in diagnosing malignant potential in tumours that have not apparently metastasized at the time of diagnosis.

The approach to the medulla will be different and will be broadened to include extra-adrenal paragangliomas. Over the past few years there have been major advances in our understanding of these tumours, and a realization that at least 30% are hereditary in nature. Genotype-phenotype correlations have been elucidated in some forms, including links to metastatic behaviour. These aspects will be included in addition to standard histological approaches.

The normal adrenal gland

The normal adult human adrenal glands sit at the upper poles of the kidneys. They weigh 4g at autopsy in cases of sudden death. They are divided from inferomedial to lateral into head, body and tail with lateral extensions – the alae. The medulla is found centrally in the head and body and may extend into the alae, but is absent from the tail. It accounts for about 10% of the weight. Where present, it lies centrally, although the border between it and the cortex is irregular. A cuff of cortex invaginates around the central vein, thus lying within medullary tissue.

The cortex plays a pivotal role in homeostasis, producing glucocorticoids, mineralocorticoids and sex steroids and is made up of three zones with different histological features. The zona glomerulosa (ZG) comprises small angular cells dispersed focally below the capsule. It produces aldosterone. The zona fasciculata (ZF) comprises large lipid-laden cells arranged in columns from the ZG or capsule to the inner zona reticularis (ZR). It is thought to be the main source of glucocorticoids (cortisol in the human gland). The cells of the ZR are eosinophilic and arranged in cords around vascular sinusoids. This zone is also able to produce cortisol and is the source of adrenal androgens. The production of aldosterone is mainly under the control of the renin-angiotensin system, although potassium, adrenocorticotrophin (ACTH), catecholamines and prostaglandins also play a role and recent evidence suggests that endothelial cell-derived factors and adipokines are involved. The production of aldosterone specifically by the ZG is because the enzyme aldosterone
synthase is expressed only in that zone. The secretion of cortisol is controlled mainly by ACTH acting through the ACTH membrane receptor, a G protein linked receptor, and cyclic AMP (cAMP). Cyclic AMP binds to the regulatory subunits of protein kinase A (PKA), influencing gene transcription. Other factors that may be involved include insulin-like growth factors (IGF-1 and IGF-2), adrenomedullin and transforming growth factor β. Activin A may have an inhibitory role and catecholamines may be involved.

**Paraganglia**

Paraganglia are neuroendocrine organs, comprising mainly cells originating in the neural crest. They secrete catecholamines or indolamines and peptides. They are divided into two groups, associated with the sympathetic or parasympathetic nervous systems. Sympathetic paraganglia lie close to the paravertebral and prevertebral ganglia in the para-axial region of the trunk or in the connective tissue adjacent to pelvic organs. The largest is the adrenal medulla. They secrete catecholamines in response to sympathetic neural stimulation. Parasympathetic paraganglia lie close to vascular structures and branches of the glossopharyngeal and vagus nerves in the head and neck. They include the carotid body. They function as chemoreceptors responding to changes in oxygen pressure in arterial blood. Both types comprise nests of neuroendocrine cells surrounded by sustentacular cells with a dendritic shape. These may be seen more easily when stained for S100 protein. The neuroendocrine cells stain positively for general neuroendocrine markers including chromogranin A and synaptophysin. They also usually stain for enzymes involved in the catecholamine biosynthetic pathway, including tyrosine hydroxylase (TH) (1).

**DISEASES OF ADRENAL CORTEX**

**Adrenal cortical hyperplasia**

**General aspects** Prolonged stimulation of adrenocortical function is often accompanied by hypertrophy and hyperplasia of cortical cells. The average adrenal weight at hospital autopsy is 6g, compared to 4g in sudden deaths, due to stimulation by ACTH associated with the stress of terminal illness. In addition, multiple nodules may be identified at autopsy in 53% of cases (2). These are usually regarded as hyperplastic. They range in size from microscopic to large nodules and may result in marked discrepancy in weight between the two glands. Their pathogenesis is unclear, but they increase with age and have been reported as being more common in patients with vascular diseases.

**Primary hyperaldosteronism** This accounts for 5-13% of cases of hypertension (3), mostly sporadic. About one third are associated with an adrenal adenoma and a further 60% with bilateral idiopathic hyperplasia of ZG (IHA). Unilateral hyperplasia accounts for ~2% of cases. Where a tumour is present the ZG may be normal or hyperplastic. Micronodules may be found. However, because the ZG comprises only a small proportion of the cortex, hyperplasia of this zone does not usually result in an overall increase in adrenal weight.

**Cushing’s syndrome** The clinical features of this syndrome are well recognized and include centripetal obesity, moon face, hypertension, striae, osteoporosis and psychiatric symptoms. Two-thirds of cases are the result of hypersecretion of ACTH by the anterior pituitary gland, with 80% to 90% of these patients having a corticotroph adenoma. Most have bilateral
diffuse cortical hyperplasia and the glands usually weigh 6-12g, with broadening of the ZF and ZR and a relative prominence of ZR. Microscopic nodules are not uncommon, especially in the outer ZF. Ten to 20% of patients have bilateral nodular hyperplasia, with nodules visible to the naked eye. The intervening cortex is also hyperplastic. The relationship between the two is unclear, but they may be a continuum, with nodules developing in longer-standing disease.

Ectopic ACTH syndrome underlies about 15% of cases, around half associated with small cell lung carcinoma or bronchial carcinoid. Other associated tumours include thymic carcinoids, neuroendocrine tumours of pancreas, medullary carcinoma of thyroid and phaeochromocytoma. The adrenals usually show bilateral symmetrical hyperplasia, weighing an average of 15g each. Nodules are rare. Compact cells extend out close to the capsule and pleomorphism and mitotic figures are not infrequent.

Fifteen to 20% of adults have an adrenal tumour, equally split between benign and malignant. Because of the increased negative feedback to the pituitary, ACTH secretion is suppressed and the adjacent and opposite ZF and ZR are atrophic. Because of this, the ZG may appear more prominent. This should not be confused with hyperplasia.

ACTH-independent macronodular adrenal hyperplasia (AIMAH) is a rare variant of the syndrome (4-6). The adrenal glands are often distorted and markedly enlarged. ACTH levels are suppressed. In many of these cases the adrenal expresses inappropriate receptors and responds to ligands such as gastric inhibitory polypeptide (GIP), where the increase in cortisol secretion is related to food intake. Other receptors identified include those for vasopressin, luteinizing hormone (7) and serotonin.

In the rare inherited Carney complex the syndrome is associated with primary pigmented nodular adrenocortical hyperplasia (PPNAD) (8). Both adrenal glands consist of multiple dark brown to black nodules, usually 1-3mm in diameter. The combined weights range from 4-21g. The intervening cortex appears suppressed.

**Virilization and feminization** Excess production of sex steroids causes virilization, feminization or precocious puberty, depending on the age and sex of the patient and the nature of the steroids secreted. Congenital adrenal hyperplasia is a group of autosomal recessive diseases associated with inherited defects in steroidogenesis (9, 10). Most present early in life. The lack of negative feedback by cortisol to the pituitary causes an increase in ACTH secretion with diversion of precursor steroids into androgen synthesis and marked adrenal cortical hyperplasia with a characteristic cerebiform appearance and lipid depletion. An unusual variant is congenital lipid hyperplasia, in which there is cholesterol accumulation in the hyperplastic gland. A few of these cases are due to mutations in the gene encoding the side chain cleavage enzyme that converts cholesterol to pregnenolone but the majority have mutations in the gene for steroid acute response (StAR) protein that transports cholesterol to the mitochondrion to start steroidogenesis (11).

**Other diseases** About 25-40% of patients with MEN1 develop adrenal disease, usually non-functional adenomas or hyperplasia. Carcinomas are rare.
ADRENAL CORTICAL TUMOURS

Adrenal cortical adenoma

Single nodules are usually regarded as adenomas, and clonal analysis shows that most are monoclonal, supporting this approach. They often weigh less than 50g but may be large. They are intra-adrenal and may be unencapsulated, have a pseudocapsule or a true capsule. Histologically they comprise predominantly lipid-laden cells resembling ZF, arranged in an alveolar pattern interspersed with short cords. Focal groups of compact cells may be seen. Mitotic activity is rare. In patients with Conn’s syndrome, there may be cells resembling ZG and others showing mixed features of ZG and ZF – ‘hybrid’ cells. Compact cells often predominate in tumours associated with virilization. This may cause problems in assessing malignant potential.

Adrenal cortical carcinoma

This is a rare but aggressive tumour, with a prevalence of between 0.5 and 2 per million. It accounts for up to 2% of all malignancies. Surgery is still the mainstay of treatment. Over half show local invasion or metastases at the time of presentation and this is associated with poorer survival; one study reporting median survival of 15 months compared to 101 months for intra-adrenal tumours. Functioning tumours account for between 24% and 74% of cases. Most weigh more than 100g although some would use 50g as the threshold for suspicion of malignancy. They usually range in size from 3cm to 40cm. Some are encapsulated, but many are adherent to, or invade, fat or adjacent organs. On slicing, they often show lobulation with fibrous bands and areas of necrosis and haemorrhage. Where metastases are present these are commonly found in liver, lung, retroperitoneum, lymph nodes and bone. Histologic examination shows a less ordered structure than in adenomas. Trabecular and diffuse patterns are common. Some may show focal or widespread myxoid change. Nuclear pleomorphism is seen and mitoses, including atypical forms, are often present. There may be confluent necrosis. Capsular and vascular invasion can often be identified and infiltration of local tissues. Malignancy is defined both by local invasion and metastasis.

Diagnosis of malignancy

While the diagnosis of malignancy is easy in most cases, all intra-adrenal tumours must be assessed histologically for malignant potential. Tumours should be widely sampled and any suspicious areas processed. The most commonly used approach is that of Weiss (12, 13). Nine histological features are assessed, and the presence of three or more indicates malignant potential. An alternative is the modified Weiss index (14). This omits histological features that had poor inter-observer correlation and incorporates the others into a weighted score. This system has performed well in diagnosing malignancy when compared to van Slooten and Weiss (15). In a few cases, the different systems may give different diagnoses, and occasionally a diagnosis of indeterminate or borderline tumour may have to be made.

A different approach has recently been proposed, starting from an analysis of the sensitivity and specificity of each of the individual Weiss features in diagnosing malignancy. Based on the observation that diffuse architecture was seen in 76% of carcinomas compared to 9% of adenomas, the group examined the reticulin network histologically. They found that disruption was 100% sensitive and 96% specific for identifying tumours with a Weiss score
≥3. The additional presence of one of the following features - mitotic rate >5 per 50 high power fields (HPF), necrosis and venous invasion – gave 100% sensitivity and specificity (16).

Proliferative activity, as defined by the Ki-67 index, is usually higher in carcinomas than in adenomas, but the threshold has varied in many of the studies. We have found in our own practice that an index greater than 5% is seen only in carcinoma. However, a low index does not define benign behaviour. Immunopositivity for p53 is seen in about 50% of carcinomas and rarely in adenomas and carcinomas overexpress insulin-like growth factor-2 (IGF-2).

The main area of difficulty in applying the Weiss criteria in adults is oncocytic tumours. Oncocytic tumours resemble those at other sites and are composed of large eosinophilic cells with accumulation of mitochondria. Originally reported as non-functioning and benign, functional and malignant variants have now been described. The problem is that most have less than 25% clear cells, pleomorphic nuclei and diffuse architecture, a combination giving a Weiss score of 3, and thus a malignant diagnosis. A modification of the Weiss approach has been suggested (17). Three major criteria and four minor criteria have been proposed and tumours are defined as malignant, of uncertain malignant potential or benign, based on the presence or absence of these. This approach has been validated in a recent study that also suggested that oncocytic carcinomas have a better outcome than usual type (18).

Molecular aspects of adrenal cortical tumours

There are a number of comprehensive reviews on this topic (19-21). Comparative genomic hybridization (CGH) and interphase cytogenetic studies have demonstrated widespread chromosomal changes in carcinomas and fewer in adenomas. Further investigations have shown loss of heterozygosity (LOH) at 2p16 (92%), 11q13 (≥90%) and 17p13 (≥85%) in carcinomas. Despite the LOH at 17p13, somatic mutations of TP53 are found in only 25-35% of sporadic adult carcinomas, suggesting another tumour suppressor gene (TSG) at this locus.

The most common abnormality in carcinoma is overexpression of IGF-2, reported in about 90% of cases. The IGF-2 gene is located at 11p15 where there are complex interactions of a number of genes. It is maternally imprinted and expressed from the paternal gene. In adrenal carcinomas, there is usually loss of the maternal allele and duplication of the paternal allele. The Wnt/β-catenin pathway is involved in a range of pathologies including PPNAD, hyperplasias, adenoma and carcinomas, so is not useful in differential diagnosis. Gene profiling studies are in agreement that there are differences between benign and malignant lesions although the molecular signatures described differ, except for the overexpression of IGF-2 in carcinoma.

There have now been a number of studies of microRNA (miR) expression in a range of adrenal cortical diseases, but the numbers of cases are still small, so the significance of the findings requires further validation (22-24). However, differences have been identified between normal adrenals and hyperplasias; also between normal and benign and malignant tumours. Upregulation of Mir483-5p has been associated with malignancy in two studies.
HYPERPLASIA AND TUMOURS OF PARAGANGLIA

Adrenal medullary hyperplasia

Adrenal medullary hyperplasia (AMH) is an increase in the number of chromaffin cells within the adrenal gland (25-27). It can be diagnosed with certainty only by morphometric analysis but in general diagnostic practise is recognized as extension of the medullary tissue into the tail or alae of the gland where it is normally absent or sparse. When assessing the relative proportions of cortex and medulla, it has to be remembered that a reduction in the volume of the cortex may give a relatively higher proportion of medulla. Hyperplasia may be diffuse or nodular or a combination of both. The distinction between nodular hyperplasia and phaeochromocytoma can be difficult, and an arbitrary cut-off point of 10mm diameter has been proposed. The demonstration of clonal lesions in both of these groups suggests that some lesions smaller than 10mm may indeed be neoplastic rather than hyperplastic. AMH is a well-recognized precursor to phaeochromocytoma in MEN 2 but does not seem to be involved in the other types.

AMH has also been described occasionally in sudden infant death syndrome, in Beckwith-Wiedemann syndrome, and in association with adrenocortical adenoma. Whether these associations are by chance or specific is unclear.

Carotid body hyperplasia

Hyperplasia is found in people living at altitude in response to the hypoxic stimulus. Hypertrophy and hyperplasia also occurs in people with chronic obstructive airways disease and in patients with cystic fibrosis and cyanotic heart disease (28-30).

Paragangliomas

The current approach defined by the World Health Organization (WHO) in 2004 is to reserve the term ‘phaeochromocytoma’ for intra-adrenal tumors and to define the others as paragangliomas of sympathetic or parasympathetic type, further defined by site (31). Thus, a phaeochromocytoma is a sympathetic paraganglioma of the adrenal gland. A carotid body tumour is a parasympathetic paraganglioma of the carotid body.

The true incidence of paragangliomas is not known, but an estimate of ~ 1/300,000 has been made. An annual incidence of between 0.4 and 9.5 per 10^7 for phaeochromocytoma has been reported and of 1 per 10^7 for paragangliomas of the head and neck. Based on the relative distribution of sites paragangliomas at other loci may have an incidence of 0.45 per 10^7.

It is now known that at least 30% of paragangliomas arise on a background of genetic mutations and the distribution and behaviour varies with the mutation (32, 33). Approximately 10 per cent of phaeochromocytomas are associated with multiple endocrine neoplasia (MEN) types 2A and 2B, von Hippel-Lindau (VHL) syndrome and neurofibromatosis type 1 (NF1) and these have been recognized for some time. About 50% of MEN2 patients develop phaeochromocytoma but extra-adrenal tumours are extremely rare. They coexist with medullary carcinoma of thyroid (MTC) and parathyroid hyperplasia in MEN2A and with MTC and mucocutaneous neuromas in MEN2B. They occur in 0.1% to 5.7% of patients with NF1 and in 10% to 30% of those with VHL disease, in whom they
characterize type 2 disease. The major increase reflects mutations recently identified in the genes encoding the subunits of succinate dehydrogenase - *SDHA, SDHB, SDHC* and *SDHD* (abbreviated together as *SDHx*) in about 20% of apparently sporadic paragangliomas, including extra-adrenal tumours. These inherited tumours appear to segregate into two groups on the basis of gene expression profiling; those with *VHL* and *SDHx* mutations (cluster 1) show high expression of hypoxia, angiogenesis and matrix related genes while those with *RET* (rearranged during transfection), *NF1* (neurofibromatosis type 1) and *TMEM127* (transmembrane protein 127) mutations (cluster 2) have changes consistent with activation of the Ras MAPK pathway, protein trafficking and other functions.

The genes involved in familial disease do not appear to play significant roles in the pathogenesis of sporadic tumours, with *RET* mutations in up to 10% and *VHL* mutations in about 4%. However, since the patterns of gene expression are similar in inherited and sporadic disease, it suggests that other genes encoding proteins in the same pathways may be involved.

**Behaviour of paragangliomas**

The vast majority of paragangliomas are benign. Malignancy is defined only by the presence of metastasis at sites where paraganglial tissue would not normally be found. It is recognised that aggressive local invasion may be lethal, but this does not correlate with metastatic potential and is not defined as malignant.

**Diagnosis of malignant potential**

A lot of effort has been put into diagnosing metastatic potential in apparently localised tumours. From early on, it was realised that intra-adrenal tumours were more often benign than those in extra-adrenal locations. This is now being linked partly to genotype-phenotype correlations in hereditary tumours.

**Histological assessment of malignancy** The absence of intracellular ‘hyaline globules’ and paucity of sustentacular cells have been reported to correlate with malignancy, but neither is sufficiently specific or sensitive in an individual tumour. A multifactorial histological approach was put forward for phaeochromocytoma (Phaeochromocytoma of the Adrenal gland Scaled Score – PASS) (34) but has not been consistently reproducible (35). Proliferation rates based on Ki-67 score also give discrepant results.

**Genetic factors** It is clear that almost all phaeochromocytomas associated with Multiple Endocrine Neoplasia type 2 (MEN2, *RET* mutations) are benign. However, in those associated with *SDHB* mutations, up to 50% have metastatic potential. Genetic testing has now become important in this area. Antibodies to SDHB, which allow the identification of all *SDHx* mutations by negative staining guide molecular testing in this area (36, 37). Positive staining guides testing to genes associated with cluster 2.

**Other factors** There is gathering evidence that biochemical testing can be helpful in the diagnosis of malignant potential. This will be discussed.
References