This article reviews published evidence on the diagnosis and classification of pituitary gland tumours and the relevance of histological and genetic features to prognosis. Much of the literature is devoted to the histological, ultrastructural, and immunocytochemical classification of pituitary adenomas (extensively supported by multicentre studies), with little consensus on the identification of prognostic features in adenomas, particularly in relation to invasion. There is a lack of correspondence between clinical and pathological criteria to identify and classify invasion, and a need to reassess the nomenclature and diagnostic criteria for invasive adenomas and carcinomas. Recent cytogenetic, genetic, and molecular biological studies have identified no consistent abnormalities in relation to pituitary tumour progression, although many genes are likely to be involved. In light of these uncertainties, an approach to the diagnosis and classification of pituitary adenomas is suggested, based on robust criteria from earlier studies and incorporating provisional data that require reassessment in large prospective studies with an adequate clinicopathological database.

Intracranial lesions arising in the region of the pituitary gland are relatively common; the most frequent of these are pituitary adenomas, which account for 6–10% of all symptomatic intracranial tumours.1 However, the pituitary gland can be involved by a wide range of other pathological processes arising both within the gland itself and in the surrounding structures: the skull in the region of the sella turcica, dura mater, blood vessels, cranial nerves, and brain.4–6 Advances in neuroimaging have increased the sensitivity of detection of small lesions in the pituitary region, which are now readily accessible to neurosurgeons following recent developments in trans-sphenoidal surgery, including microsurgery and endoscopic resection of small intrasellar lesions.5,6,8 Consequently, pathologists now encounter an increasing number of lesions for diagnosis both within and around the pituitary gland, which comprise an ever-widening spectrum of pathological processes.1,2 The aim of this article is to provide a brief review of the clinical features of lesions that are encountered from within and around the pituitary gland, to assess current diagnostic pathological techniques, and to explore the expanding evidence base for the classification of pituitary adenomas and the clinical relevance of these approaches.

**OVERVIEW OF PITUITARY REGION PATHOLOGY AND CLINICAL SYMPTOMATOLOGY**

The pituitary gland can be affected by a wide range of lesions, which may arise either within the gland itself or its surrounding structures (table 1).2,4,6,8 These lesions all tend to present with clinical symptoms and signs that relate either to endocrine dysfunction of the pituitary gland, the local effects of an expanding mass in the region of the pituitary gland on adjacent structures (particularly the optic nerves and chiasm), or the non-specific effects of an expanding intracranial mass associated with raised intracranial pressure.2 The clinical symptoms are of course also dependent on the underlying nature of the lesion, its size and extension beyond the sella turcica, and the consequent effects on adjacent structures beyond this region.

"Larger lesions can produce signs and symptoms of raised intracranial pressure, of which headache is the most common manifestation"

Small lesions confined to the pituitary gland may be asymptomatic (see below for “silent” adenomas), or in the case of functioning adenomas will present with signs and symptoms of endocrine dysfunction (either excess or reduced production of one or several anterior pituitary hormones), particularly in younger patients.1,3,4 Clinical biochemistry laboratories in centres with an endocrine clinic are able to provide rapid and comprehensive assays for pituitary hormones and will also help with pituitary suppression or stimulation tests. Larger lesions with suprasellar

**Abbreviations:** ACTH, adrenocorticotropic hormone; 1,24, ACTH, adrenocorticotropic hormone; Ct, computed tomography; FGF, fibroblast growth factor; GH, growth hormone; MEN-1, multiple endocrine neoplasia type 1; MRI, magnetic resonance imaging; PRL, prolactin; ptd-FGFR-4, pituitary tumour derived N terminally truncated isoform of fibroblast growth factor receptor-4; PTTG, pituitary tumour transforming gene; TSH, thyroid stimulating hormone
INTROOPERATIVE DIAGNOSIS AND SPECIMEN HANDLING

Although many pituitary adenomas can be diagnosed after clinical, biochemical, and radiological investigations (and small intrasellar tumours producing PRL or growth hormone (GH) may be treated medically if there are no other indications for surgery), neurosurgical management will often require intraoperative diagnosis to confirm the presence of a tumour in the resected tissue. This can help at the time of surgery to distinguish adenomatous from non-adenomatous pituitary tissue, and to ensure that for larger lesions extending beyond the sella turcica no other type of neoplasm is present for which further neurosurgical procedures may be required. Intraoperative diagnosis for pituitary adenomas is readily achievable on smear preparations alone, which can achieve a high degree of diagnostic accuracy.11–15 Smear preparations have several advantages in that they require the use of only small (1 mm²) portions of tissue, they can be prepared and stained rapidly using a variety of stains (of which the most common are toluidine blue and haematoxylin and eosin), and their preparation requires no specialised facilities or equipment other than access to a class 1 hood in which to handle the unfixed tissue. The preparation of smears from pituitary adenomas is relatively easy, because they usually have a soft consistency that readily allows a monolayer preparation to be made, which is ideal for cytological examination (fig 1).11,13 Smear preparations of pituitary adenomas allow a ready distinction from the non-adenomatous gland in terms of the nuclear pleomorphism and relatively uniform cytological characteristics seen in adenomas, and the identification of other pathological processes, such as inflammatory disorders or other forms of neoplasms, such as metastatic carcinoma or meningioma (fig 1). Certain specific cytological features may be identified in haematoxylin and eosin stained smear preparations that suggest a specific type of pituitary adenoma—for example, the paranuclear fibrous bodies in sparsely granulated growth hormone adenomas.
Intraoperative diagnosis for pituitary adenomas is readily achievable on smear preparations alone, which can achieve a high degree of diagnostic accuracy.

Smear preparations can be usefully supplemented by cryostat section diagnosis, particularly if the differential diagnosis includes other types of lesions such as schwannoma or craniopharyngioma, which may be tough in consistency and therefore difficult to smear. Rapid reticulin staining of a cryostat section may be useful in selected cases, particularly if there is doubt as to whether the tissue examined is normal anterior pituitary tissue or an adenoma, because adenomas lack the characteristic reticulin framework of normal anterior pituitary tissue. It has been suggested that the use of smear preparations for intraoperative diagnosis can achieve a higher degree of diagnostic accuracy than cryostat sections, but both approaches have their limitations in terms of assessment of adequacy of local resection. Furthermore, the use of tissue for cryostat sections can compromise the quality of the final histology obtained on the specimen, which can result in subsequent difficulties in histological interpretation and diagnosis. Therefore, cryostat sections for intraoperative diagnosis are best avoided in certain circumstances—for example, in specimens from patients with Cushings disease in whom no identifiable lesion is present on MRI scans. At the time of intraoperative diagnosis it is usually possible to store a piece of unfixed tumour tissue in a freezer, which can be used either for supplementary biochemical studies concerning hormone production, or for DNA or RNA extraction and analysis. It is also recommended that a small portion of tissue is placed into a fixative for electron microscopy, because ultrastructural examination is essential for the diagnosis of certain rare subtypes of pituitary adenoma.

PITUITARY ADENOMAS

These are by far the most common tumours arising in the pituitary gland and their pathology is extensively reviewed elsewhere. Rather than a repetitious listing of these well described lesions, this article will focus on the need to distinguish subtypes of pituitary adenomas that are of particular clinical relevance. The evidence base for the histological distinction of adenomas from nodular hyperplasia, invasive or atypical adenomas, and carcinomas will be reviewed. Table summarises the clinicopathological features and relative incidence of pituitary adenomas. Pituitary adenomas occur across a wide age spectrum, from childhood and adolescence to old age. As noted above, it has been estimated from both clinical and postmortem studies that pituitary adenomas occur in around 20% of the normal population, but only a minority of these are symptomatic. Adenomas may rarely arise in ectopic pituitary tissue in the cranial cavity or bony sinuses, and have been described within an ovarian teratoma as an exceptional finding. Multiple adenomas (including those of different cell types) have occasionally been reported, usually in patients with Cushings disease. Other apparently unrelated pathological processes occasionally coexist within a pituitary adenoma, including sarcoidosis, toxoplasma infection, and metastatic carcinoma.

The classification of pituitary adenomas is based on a four step process, as follows:

1. Functional classification, to define these tumours in terms of their endocrine activity, noting that a large group of adenomas are clinically non-functional or endocrinologically inactive.

2. Anatomical and neuroradiological classification, based on tumour size and the degree of local invasion. The most widely
used classification is based on that by Hardy, which places adenomas into four grades (table 3). As part of this assessment, it is important to understand that local invasion in certain subtypes of pituitary adenomas is relatively common, although not necessarily indicative of malignant potential. Histological classification, firmly based on the immunocytochemical characterisation of the tumours in terms of hormone production, including the six major anterior pituitary hormones (adrenocorticotrophic hormone (ACTH), follicle stimulating hormone, GH, luteinising hormone, PRL, and thyroid stimulating hormone (TSH) and the α subunit of glycoprotein hormones. Other antibodies can be used to identify certain subtypes of pituitary adenoma—for example, the use of antibodies to low molecular weight cytokeratins to label the filrous bodies in sparsely granulated GH adenomas. Older classifications of adenomas based on tinctorial stains are obsolete and should be abandoned.

Ultrastructural classification, which can confirm that non-functional lesions are of pituitary origin, and characterises the cytological differentiation of tumour cells in terms of anterior pituitary cell types.

Histological classification, firmly based on the immunocytochemical characterisation of the tumours in terms of hormone production, including the six major anterior pituitary hormones (adrenocorticotrophic hormone (ACTH), follicle stimulating hormone, GH, luteinising hormone, PRL, and thyroid stimulating hormone (TSH) and the α subunit of glycoprotein hormones. Other antibodies can be used to identify certain subtypes of pituitary adenoma—for example, the use of antibodies to low molecular weight cytokeratins to label the filrous bodies in sparsely granulated GH adenomas. Older classifications of adenomas based on tinctorial stains are obsolete and should be abandoned.

INVASIVE PITUITARY ADENOMAS
The apparent contradiction in nomenclature of an invasive benign tumour is not confined to pituitary adenomas alone, but this topic is of particular importance in view of the relation of the pituitary gland to crucial intracranial structures. Pituitary adenomas lack a true capsule, and their relation to the surrounding normal anterior pituitary tissue can be difficult to assess, particularly in fragmented biopsy specimens. More important than invasion of the surrounding gland is the propensity of adenomas to invade surrounding structures. Sampling of the dura mater of the diaphragma sellae found evidence of invasion in 66% of Hardy grade I tumours, which rose to over 90% for grade III tumours in one series. In a more recent larger study, dural invasion was found in 45% of all cases, with a higher frequency in patients undergoing repeated trans-sphenoidal surgery and for those patients with macroadenomas (> 40 mm). The identification of dural invasion indicates that an adenoma may be incompletely resected, and there was a significantly decreased survival rate in patients with invasive adenomas in this study.

“More important than invasion of the surrounding gland is the propensity of adenomas to invade surrounding structures”

Because dura mater sampling is not routinely performed in pituitary surgery, the identification of invasion generally depends on neuroradiological and neurosurgical assessment (see above). It is established that certain tumour types are more likely to be invasive, including macroadenomas, non-functioning adenomas (particularly silent ACTH adenomas and silent subtype 3 adenomas), and TSH adenomas. The identification of histological criteria related to invasiveness has not been the subject of a standardised prospective assessment, but there is general agreement that “brisk” mitotic activity is associated with aggressive
growth. Although early studies showed no relation between tumour ploidy and invasion, there is an association between macroscopic invasion and the proportion of cells in the S phase of the cell cycle. This relation has been confirmed in immunocytochemical assessment of cell proliferation indices using the Ki-67 and MIB-1 antibodies. A threshold Ki-67 labelling index of 3% distinguished aggressive tumours from non-invasive adenomas with 97% specificity, with positive and negative predictive values of 96% and 80%, respectively, in one series.

No consistent relation between tumour invasion and nucleolus organiser regions in adenoma cells has been identified. Although immunoreactivity for p53 is absent in non-invasive adenomas, it is present in some, but not all, invasive adenomas and pituitary carcinomas (including metastatic deposits). It has also been suggested that immunoreactivity for other proteins, including hst, heat shock protein 27, and interleukin 6, may also be useful in the identification of invasive adenomas. Further assessments are required on larger prospective series to investigate these and other potential markers for invasive adenomas. Genetic events that may be associated with invasion in pituitary adenomas are summarised below.

**PITUITARY CARCINOMAS**

These extremely rare malignant tumours are identified on the basis of their capacity to metastasise via the cerebrospinal fluid pathway or to extracranial tissues. Brain invasion is not yet considered a criterion for malignancy, but it is likely that this possibility will be reassessed as neuroradiological techniques for the identification of brain invasion improve and that the practicality of brain sampling for the identification of invasion is reconsidered by neurosurgeons (as it has been for the identification of invasive meningiomas). Pituitary carcinomas are usually endocrinologically functional, with ACTH and PRL producing tumours being the most frequent. Carcinomas show a variable degree of nuclear atypia and cellular pleomorphism, but with significantly higher mitotic rates and cell proliferation indices than adenomas. Vascular endothelial proliferation and necrosis are uncommon features; immunoreactivity for p53 has been found in some metastatic deposits, but the primary tumours show a much lower incidence of immunoreactivity. As for invasive adenomas, it appears at present that there are no consistently expressed cellular markers of aggressive biological behaviour for pituitary carcinomas.

**PITUITARY HYPERPLASIA**

Physiological pituitary hyperplasia occurs in pregnancy and lactation, and primarily involves PRL cells. Non-neoplastic hyperplasia of individual pituitary cell types is an uncommon cause of excess hormone secretion. Two main patterns of pituitary cell hyperplasia may be encountered: diffuse and nodular; these may occur either singly or in combination. Diffuse hyperplasia does not greatly alter the acinar structure of the gland and thus may be difficult to detect in a fragmented biopsy specimen, particularly because there is a considerable degree of variation in the distribution of cell types in the normal anterior pituitary gland. The most clinically important form of pituitary hyperplasia is nodular hyperplasia, which most often involves a single cell type and is more likely to be associated with clinical features as a result of excess endocrine activity, mimicking a functional adenoma. All cell types can be involved, but PRL hyperplasia is the most common form, and can occasionally result from longstanding pituitary stalk compression by a pre-existing adenoma or another adjacent mass lesion. Reticulin stains are helpful in the assessment of nodular hyperplasia, because the abnormally expanded acinar structures are not uniformly distributed throughout the gland and they may become confluent, resulting in disruption of the normal reticulin framework.

**GENETIC STUDIES IN PITUITARY TUMOURS**

There is much evidence to support the notion that pituitary tumours are clonal lesions caused by intrinsic pituitary cell defects, most of which are based on X chromosome inactivation. In support of this concept, it is extremely unusual to find hyperplasia of the anterior pituitary gland surrounding an adenoma (which might be expected if the adenoma arose as the result of an external hormonal stimulus), although occasional examples have been described. Multiple synchronous adenomas have been described in the pituitary gland, including tumours of different cell types, although they are extremely rare. A multiclonal origin has been proposed for these tumours, and there is increasing evidence to suggest that at least some sporadic tumours may also be multiclonal.

“Deletions in the region of 13q14 have been identified in pituitary adenomas, suggesting the presence of a tumour suppressor gene at this locus.”

Genetic studies of pituitary adenomas have revealed that most of the mutations that have been identified in other malignancies are usually absent, and the molecular events leading to adenoma formation are still poorly understood. Although pituitary adenomas occur in multiple endocrine neoplasia type 1 (MEN-1), loss of heterozygosity at the MEN-1 gene locus is uncommon in sporadic adenomas. Similarly, although pituitary GH adenomas occur as part of the Carney complex, one of the genes responsible, PRKAR1A, does not appear to be involved in sporadic pituitary tumours. Deletions in the region of 13q14 have been identified in pituitary adenomas, suggesting the presence of a tumour suppressor gene at this locus. It has also been suggested that amplification of the HRAS and CMYC genes and inactivation of the tumour suppressor genes RB1, TP53, and NM23 may represent mechanisms by which pituitary tumours progress, but there is as yet no evidence of their consistent involvement in pituitary adenoma invasion or in pituitary carcinomas. Certain cell specific genetic abnormalities have been identified—for example, up to 40% of GH adenomas have GSP mutations, resulting in activation of the Gs α subunit. Inactivation of p16 has been identified in up to 80% of adenomas (particularly in large tumours) in two recent studies, possibly as a result of CDKN2A methylation. A novel oncogene, pituitary tumour transforming gene (PTTG), is overexpressed in a wide range of pituitary adenomas and immunocytochemistry for PTTG protein is positive in most adenomas, but absent in normal pituitary cells. The precise role of PTTG in oncogenesis is uncertain: PTTG may interact with fibroblast growth factor (FGF) to stimulate vascular growth, or it may activate p53 to cause apoptosis. A recent large study found an association between cyclin D1 genotype (CCND1) and tumour grade in sporadic pituitary adenomas, but the clinical relevance of this finding remains uncertain until it is reproduced in other populations with these tumours. A novel pituitary tumour derived, N terminally truncated isoform of FGF receptor-4, ptd-FGFR4, has been identified recently. ptd-FGFR4 is not expressed in normal pituitary tissue, and has a distinctive cytoplasmic residence. It is transforming both in vivo and in vitro, and targeted expression of ptdFGFR4 in transgenic mice results in pituitary tumours that morphologically resemble PRL adenomas in humans, in the absence of PRL cell hyperplasia.

**OTHER TUMOURS ARISING IN THE PITUITARY GLAND**

Table 1 summarises the other tumours that arise in the pituitary gland. Many of these, particularly the cystic lesions such as craniopharyngioma, meningiomas, and germ cell

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tumours are extensively described in the literature and generally do not pose major diagnostic problems. Pituitary adenomas (regardless of cell type) are characterised by the expression of synaptophysin, neuron specific enolase, low molecular weight cytokeratins, and PTTG. However, tumours arising in the neurohypophysis are uncommon and can represent a source of diagnostic difficulty, which is particularly important in terms of implications for patient management. The most common tumour arising in the neurohypophysis is the granular cell tumour, which is occasionally encountered as an incidental finding at necropsy. These tumours resemble granular cell tumours arising in other parts of the body. Their histogenesis is uncertain; they occur in adults (usually in late middle age) and occasional examples have been reported in association with endocrine syndromes, including acromegaly. Most are benign, but occasional cases exhibit local infiltration or recur after surgery. Pituicytomas are extremely uncommon tumours of glial lineage, which occur in adults and are curable by total resection. These benign tumours are thought to be derived from neurohypophyseal pituicytes, and are characterised by the expression of S-100 protein and vimentin, with variable positivity for glial fibrillary acidic protein. Although not associated with MEN-1, a case has been described that occurred in a patient with multiple endocrine tumours.

Other rare primary pituitary tumours include gangliogliomas and gangliocytomas, which can be associated with endocrine secretion. It has been suggested that some of these lesions may represent examples of transdifferentiation, rather than a primary neuronal neoplasm of the neurohypophysis. Other even rarer tumours arising in the pituitary gland include lymphomas, astrocyomas (including pilocytic astrocytomas and pleomorphic xanthoastrocytomas), ependymomas, and the recent entities of spindle cell oncocytoma and the rather poorly defined suprasellar monomorphous pilomyxoid neoplasm. The pituitary gland may also be involved in a wide range of non-neoplastic cystic lesions and inflammatory infectious disorders, which may present with clinical features similar to those of a non-functioning adenoma; these lesions are summarised in table 1.

CONCLUSIONS

The pathologist has a key role to play in the multidisciplinary team dealing with patients with pituitary region tumours. An accurate pathological diagnosis of these lesions can only be achieved if the pathologist is in close cooperation with clinicians dealing with the patient, so that full clinical details (including medical treatment, biochemical, and neuroradiological findings) are provided on the consultation request form; this is particularly important if an intraoperative diagnosis is requested. The adequate pathological assessment of pituitary adenomas requires extensive immunocytochemistry and, in some cases, electron microscopy. Based on available evidence, a diagnostic approach for pituitary adenomas is outlined in table 4, which includes prognostic indicators in addition to markers of cytodifferentiation. It is inevitable that this approach will require further modification as additional data emerge on the genetic abnormalities associated with pituitary tumour pathogenesis. As this genetic data improves, it is anticipated that the relation between pituitary adenomas, invasive adenomas, and carcinomas will be clarified, allowing greater diagnostic accuracy and the provision of prognostically useful information.

ACKNOWLEDGEMENTS

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Table 4  Approach to the investigation and diagnosis of pituitary adenomas

<table>
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<th>Intraoperative diagnosis</th>
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<td>Cryostat section</td>
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<td>Paraffin wax sections</td>
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<td>Routine diagnosis</td>
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<td>Prognostic markers</td>
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<td>Synaptophysin, neuron specific enolase, low molecular weight</td>
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<tr>
<td>Genetic studies*</td>
<td>p53*, hst*, hsp 27*, and IL-6*</td>
</tr>
</tbody>
</table>

*Provisional data to be confirmed in future studies.

ACTH, adrenocorticotropic; FSH, follicle stimulating hormone; hsp, heat shock protein; IL, interleukin; LH, luteinising hormone; PRL, prolactin; TSH, thyroid stimulating hormone.
Winds of change
There once lived a pathologist by name Joe Pearson,
Like the back of his hand, he knew every lesion.
He knew them all; he'd seen them all,
Like the back of his hand, he knew every lesion.

There once lived a pathologist by name Joe Pearson,
A poem for pathology ...........................................................

Till one day, the curtain of wax fell.
He'd made a mistake, which was certainly fatal—
putting a newborn through the gates of hell;
He was left a loner in his ivory tower,
Deprived of his glory, shorn of his power.

Our good old man had but one vice,
Never a new thought could he entertain,
The winds of change—he treated with disdain.
Stagnant and resistant, the frog-in-a-well,