Received: 14 February 2024 | Revised: 21 June 2024 | Accepted: 1 July 2024

DOI: 10.1111/cyt.13418

IMAGES IN CYTOLOGY

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Cytological features of ependymal and choroid plexus tumours

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Abstract

Ependymal and choroid plexus tumours arise in anatomically related regions. Their intraoperative differential diagnosis is large and depends on factors such as age, tumour site and clinical presentation. Squash cytology can provide valuable information in this context. Cytological features of conventional ependymomas, subependymomas and myxopapillary ependymomas as well as choroid plexus tumours are reviewed and illustrated. Differential diagnostic considerations integrating morphological and clinical information are discussed.

KEYWORDS
choroid plexus tumours, ependymoma, intraoperative diagnosis, squash cytology

1 | INTRODUCTION

Ependymomas and choroid plexus tumours arise in anatomically related regions and have characteristic morphological features that generally differentiate them from other CNS tumours. While the knowledge about the biology of choroid plexus tumours has been relatively stable over the past decade, our understanding of ependymal tumours has been conceptually reshaped. Comprehensive genetic analyses have demonstrated that ependymomas fall into specific subtypes limited to specific compartments (supratentorial, infratentorial and spinal) and characterized by different clinical features and prognosis, while morphological features are often overlapping.[1] Interestingly, these molecular findings corroborated or recapitulated a number of classical morphological observations such as the occurrence of tumours with hybrid ependymoma/subependymoma morphology in the posterior fossa, which is in hindsight explained by the fact that posterior fossa tumours with ependymoma, subependymoma or indeed mixed morphology form a single cluster upon methylation analysis.[2] Likewise, classical or tanycytic ependymomas of the lumbosacral spinal cord were shown to share the methylation cluster with myxopapillary ependymomas, which are morphologically different, but arise in the same anatomical region.[3] Yet, the prognostic impact of such epigenetic similarities, especially in terms of the tendency to metastasize via the cerebrospinal fluid (CSF), a feature of myxopapillary ependymomas, remains to be defined.

It is generally believed that methylation-defined subtypes provide a strong prognostic impact in ependymomas, beyond morphological features and specifically morphological grading seems to have limited prognostic value.

Because of these limitations of pure histological analysis in ependymomas, intraoperative consultation and specifically intraoperative cytology will in many instances not be able to provide detailed prognostic information, but will help to distinguish between ependymomas and other histological tumour types. In this regard, here we review the cytopathological features of ependymal and choroid plexus tumours.

2 | EPENDYMOMA

The 2021 WHO classification of CNS tumours[4] adopts a combined morphological and molecular approach for the definition of ependymal tumour types (Table 1), reflecting their epigenetic and site-specific variation.[1] Essentially all published information on cytological features of ependymal tumours either in textbook format[5–7] or in the form of case series[8] predates the molecular era, so it remains unclear to which extent molecularly defined ependymoma types may have specific cytological features. That said, smear preparations continue to carry valuable morphological information in the intraoperative setting and are an important part of the routine pathological workup.

Smears of ependymomas are typically characterized by the following features (Figure 1A–C):

- Moderately cellular smears.
- Fibrillary matrix, often with cellular processes that are more plump than in diffuse astrocytomas.
- Perivascular pseudorosettes (often seen indirectly in the form of dispersed cells along a central vessel) or more rarely true ependymal rosettes.
- Hyalinized vessels.[9]
- Oval to round nuclei with a coarse chromatin (often resembling a 'pepper-and-salt' chromatin), usually with less nuclear pleomorphism than in diffuse astrocytomas.

Architectural features are often more difficult to identify on smears, so an additional frozen section is useful.

In some cases, cytological features of anaplasia, most notably numerous mitotic features, may be seen, but the overall value of morphology-based grading in ependymomas is limited due to the important prognostic impact of the molecular subtypes.[1] A correct intraoperative diagnosis may be expected to be more difficult in morphological variants of ependymoma. Tanycytic ependymoma, one of the more common morphological variants, which is characterized by a predominance of spindle cells mimicking Schwannoma and a predilection for the spinal cord, may nevertheless show recognizable features of ependymal differentiation in cytology.[10] The same may be true for rare morphological variants such as giant cell ependymoma.[11]

The differential diagnosis of ependymoma is large and will depend on age, tumour site, imaging and intraoperative surgical findings as well as the cyto- and histomorphological features of a given case. Typically, it may include other tumours with similar sites of predilection, such as central neurocytoma, intraventricular meningioma or, in the case of fourth ventricular tumours, pilocytic astrocytoma or medulloblastoma. A close discussion with the operating neurosurgeon may be essential for a clinically useful intraoperative consultation, both in order to obtain a maximum of information on the clinical presentation and to understand the possible implications of the intraoperative diagnosis on the neurosurgical approach.

TABLE 1 Ependymal tumours recognized by the 2021 WHO Classification.[4]

Supratentorial ependymoma
Supratentorial ependymoma, ZFTA fusion-positive
Supratentorial ependymoma, YAP1 fusion-positive
Posterior fossa ependymoma
Posterior fossa Group A (PFA) ependymoma
Posterior fossa Group B (PFB) ependymoma
Spinal ependymoma
Spinal ependymoma, MYCN-amplified
Myxopapillary ependymoma
Subependymoma

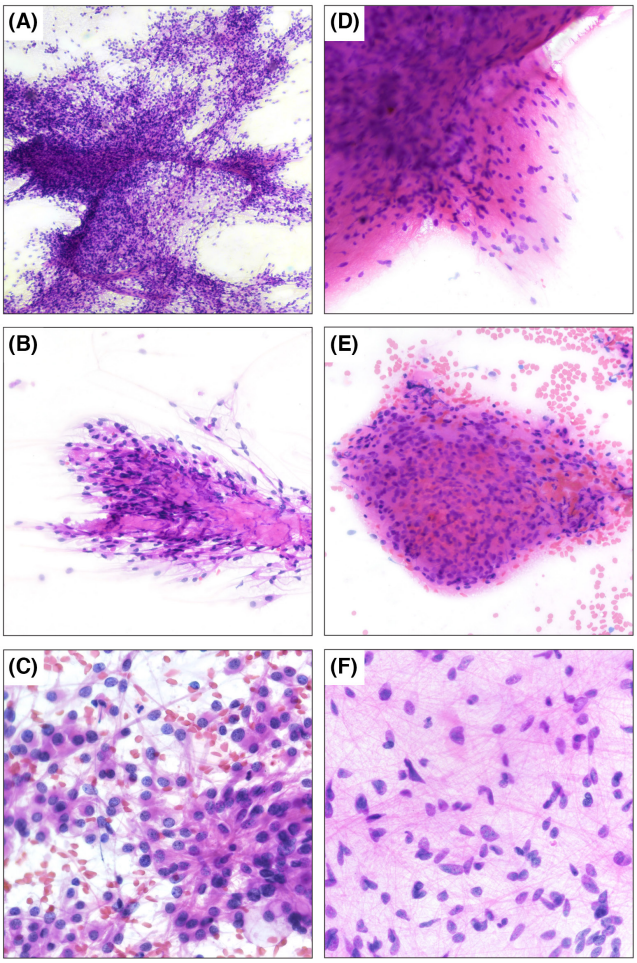

FIGURE 1 Ependymomas (A–C) often result in cellular smears. Perivascular pseudorosettes translate into tumour cells radially spreading from vessels (A, B). Nuclei are usually monotonous and round to oval, with a pepper-and-salt-like chromatin, cytoplasmic processes tend to be plump and tumour cells may have a vaguely epithelioid appearance (C). Due to their firm consistency, subependymomas (D–F) often do not smear well. If a squash cytology can be produced, fragments are often thick (A) and cytological features are more easily assessed in their periphery (E). At high magnification, small, bland nuclei in a dense, fibrillary matrix can be observed (F).

3 | SUBEPENDYMOMA

Subependymomas are slowly growing tumours that are often incidental imaging or autopsy findings,[12] but may become symptomatic and therefore undergo resection. The most informative aspect of squash cytology may be the fact that the tumour tissue does not smear well or at all, due to its firm consistency. If a smear can be obtained, cytological features include the following (Figure 1D–F):

- Low cellularity.
- Small, monotonous nuclei.
- Dense, fibrillary matrix.
- In some cases, microcystic transformation with prominent metachormatic myxoid material in Giemsa stained smears.[9]

While tumours with subependymoma morphology occur in the supra- and infratentorial compartment as well as the spinal cord, they belong to different epigenetic types; the current WHO classification nevertheless considers them a single tumour type. In the infratentorial compartment, tumours with a hybrid ependymoma/subependymoma morphology may occur that epigenetically cluster with subependymomas. Depending on sampling of the different components this may represent a pitfall for intraoperative differential diagnosis.

4 | MYXOPAPILLARY EPENDYMOMA

Myxopapillary ependymoma is a unique tumour type arising from the conus medullaris or the filum terminale; in these sites, it is the most common tumour type. While it is characterized by a slow growth, some tumours may develop extensive leptomeningeal dissemination to the effect that it may also be encountered in other CNS locations. Of note, myxopapillary ependymomas have been associated with CNS WHO Grade 2 in the classification CNS WHO5 (2021) and not with Grade 1 as in previous classifications. In its classical location, the most relevant differential diagnoses include spinal paraganglioma and schwannoma.

The cytological features of myxopapillary ependymoma reflect its histological variability and include the following (Figure 2A–F):

- Elongated cells arranged around a stroma axis.
- Monotonous medium-sized nuclei.
- Numerous blood vessels.

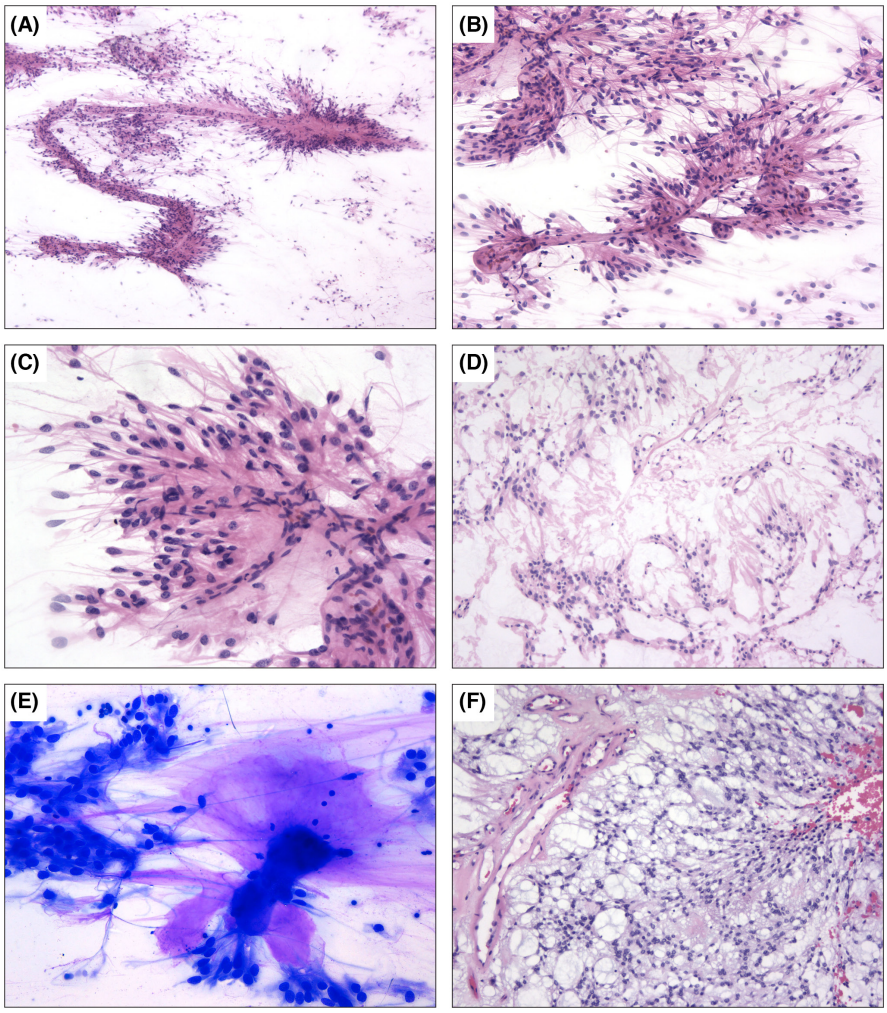

FIGURE 2 Cytological features of myxopapillary ependymoma reflect their histological heterogeneity with variable proportions of typical ependymoma-like features, papillary architecture and myxoid matrix. Cellular areas (A–C) may resemble conventional ependymoma with elongated cells radiating from a central axis, and monotonous round to oval nuclei. Frozen sections may be less demonstrative due to freezing artefacts (D). The myxoid matrix may be more readily identified on Romanowsky-stained smears (E). Matrix production and papillary features are readily recognizable on standard HE paraffin sections (F). Image E kindly provided by Dr José A. Jiménez Heffernan.

- Presence of myxoid material (more easily appreciated on Romanowsky-type stainings due to its metachromatic properties).

5 | CHOROID PLEXUS TUMOURS

The 2021 WHO classification of CNS tumours[4] recognizes choroid plexus papillomas and carcinomas (Table 2), the former being far more frequent. Choroid plexus papillomas arise in the locations of the choroid plexus, that is, the lateral ventricles or the fourth ventricle. By analogy to 'Bochdalek's flower basket'—an extension of the choroid plexus through the foramen of Luschka—they may also occur in the cerebellopontine angle.

Given their true epithelial nature, cytological findings are usually distinctive (Figure 3A–F):

- True epithelial papillae with a slender fibrovascular core and a single layer of cuboidal to cylindrical cells with monotonous nuclei
- Flat sheets of tumour cells with regular honeycomb pattern as well as isolated tumour cells.

In comparison with normal choroid plexus, cellularity is higher and architecture is more complex. Reliable distinction from atypical choroid plexus papilloma will not usually be possible, but the latter might be suspected in the presence of rare mitotic figures.

Due to its epithelial nature, distinction from other primary CNS tumours arising in the same locations, will usually be straightforward. Distinction from metastatic carcinoma will rarely be challenging either, given the lack of nuclear pleomorphism or necrosis in choroid plexus papilloma. Papillary ependymoma or papillary tumour the pineal region[13] may be considered, but will usually show at least some morphological features allowing not to mistake them for choroid plexus papilloma (e.g., presence of rosettes, lack of true epithelial papillae with cuboidal or cylindrical cell lining).

Choroid plexus carcinoma is a rare and highly aggressive tumour type mostly occurring in infants. While morphological features remain an essential part of the definition of choroid plexus carcinoma, recent molecular data suggest that the border between (atypical) plexus papilloma and choroid plexus carcinoma is not entirely clear-cut.[14]

While there is only limited published data on the cytomorphology of choroid plexus carcinoma in squash cytology,[15] it will generally be expected to reflect its histological features, that is, an epithelial nature and all or most of the defining criteria according to the WHO classification, that is, frequent mitoses, increased cellularity, nuclear pleomorphism, blurring of the papillary pattern and necrosis. Correct diagnosis will heavily depend on appropriate clinical information.

TABLE 2 Choroid plexus tumours recognized by the 2021 WHO Classification.[4]

Choroid plexus papilloma
Atypical choroid plexus papilloma
Choroid plexus carcinoma

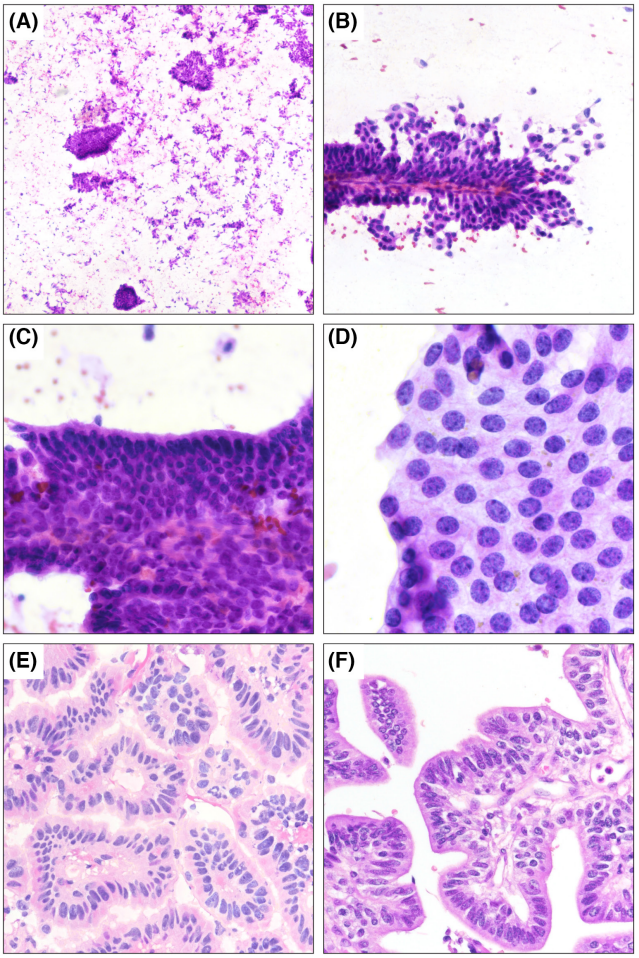

FIGURE 3 Choroid plexus papillomas are unique among primary CNS tumours due to their true epithelial nature. At low magnification (A), epithelial clusters and isolated cells are seen. Papillae are readily identified (B). Depending on the orientation, tumour cells may form a typical honeycomb pattern or cylindrical cells with well-aligned nuclei may be seen (C). At high magnification, monotonous nuclear morphology can be appreciated (D). In this case, small amounts of pigment can be identified. The essential diagnostic clue for distinction from normal choroid plexus is the more complex architecture, which is more easily seen on frozen (E) or paraffin sections (F).

6 | CEREBROSPINAL FLUID-BASED DIAGNOSIS

Due to their localization, ependymal and choroid plexus tumours may be identifiable in cerebrospinal fluid (CSF) specimens. In a retrospective study of CSF specimens positive for primary nonhematologic

central nervous system tumours, choroid plexus tumours and ependymomas each accounted for 3% of patients.[16] In this study, ependymomas were reported to cell aggregates with vague rosette formations or small three-dimensional clusters. The presence of variably sized tissue fragments with papillary architecture or rosette formation was described for choroid plexus carcinoma.

A recent study suggests that sufficient amounts of circulating tumour DNA (ctDNA) may be detectable in a substantial proportion of CSF samples (including ependymoma) in order to correctly identify the underlying tumour type through a combination of methylation analysis and copy number variations.[17] If corroborated in further studies, this may have a significant impact on preoperative diagnosis and postoperative follow-up of ependymal tumours.

AUTHOR CONTRIBUTIONS

Both authors have written the manuscript and provided microscopic images.

ACKNOWLEDGEMENTS

The authors are grateful for Dr José A. Jiménez Heffernan for kindly providing the image for Figure 2E. Open access funding provided by Universite de Geneve.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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How to cite this article: Egervari K, Hewer E. Cytological features of ependymal and choroid plexus tumours. *Cytopathology*. 2024;35:556-560. doi:10.1111/cyt.13418