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Correlative study of squash smear cytology with histopathology in a rare case of anaplastic giant cell ependymoma of the pineal

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Summary. Anaplastic giant cell ependymoma (AGCE) is a very rare neoplasm. Its cytological features, helpful for the intraoperative diagnosis, have been reported only once. AGCE is characterized by giant cells with intranuclear inclusions, besides other findings, observable in ependymal neoplasms, such as intracytoplasmic vacuoles, epithelial and glial features of the tumor cells and ependymal pseudorosettes. These findings can be detected also in intraoperative squash smear. Herein we describe a pineal AGCE, highlighting the cytological and histological correlations and underlining some useful diagnostic clues of this unusual entity.

Key words: Anaplastic giant cell ependymoma, Pineal, Cytology, Histology

Introduction

Giant cell ependymoma is a rare entity; with only 24 cases reported to date (Karabagli et al., 2011; Koh et al., 2012; Li et al., 2012). The first two cases were described by Zec in 1996 (Zec et al., 1996). To date, no cases of GCE have been reported in the pineal region where conventional ependymomas also rarely occur (Hyun et al., 2007). GCE histological grade ranges from low to high, with anaplastic features seen in less than half of the cases and usually associated with a supratentorial location (Brown et al., 1998; Pimentel et al., 2001; Moritani et al., 2003; Jeon et al., 2004; Adamek et al., 2008; Sangoi et al., 2008; Shamji et al., 2009; Dahlback et al., 2011; Koh et al., 2012; Li et al, 2012). To our knowledge, there is only one report illustrating the cytologic features of an AGCE occurring in the right temporal-occipital area (Koh et al., 2012). We report a case of AGCE that exceptionally occurred in the pineal region. Correlations between its cytological and histological features are highlighted.

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Materials and methods

A 9-year-old boy presented to our neurology department with signs and symptoms of intracranial hypertension, rhinorrhoea, diplopia and decreased visual acuity. A pineal mass was demonstrated by magnetic resonance imaging. There was no liquoral spreading of the neoplasm. The patient was immediately referred to neurosurgery, and an intraoperative diagnosis was requested. Two small fragments of the lesion were squashed and smeared, as is done in most central nervous system tumor intraoperative examinations. Smears were immediately fixed in absolute alcohol and stained with haematoxylin and eosin. After the intraoperative diagnosis, the tumor was removed. However, an extensive resection was not achieved, in order to minimise damage to the neighboring blood vessels and the brainstem. Tumor fragments were fixed in 10% buffered formalin and embedded in paraffin. One section was stained with hematoxylin-eosin, whilst others were immunostained. The following antibodies were used: GFAP (6F2; dil. 1:50, DAKO, Milan, Italy);

EMA (E29, dil. 1:50, DAKO); S-100 (polyclonal Ab; dil.1:100, DAKO); p53 (D0-1; dil.1: 50, Menarini, Florence, Italy); EGFR (31G7; dil.1:50, Zymed, Milan, Italy); pan-cytokeratin AEI/AE3 (dil.1: 100, Bio Optica, Milan, Italy); Neurofilaments (clone2F11; dil.1:50, DAKO); CD99 (2E7 MIC-2; dil.1:50, DAKO); Ki-67 (MIB-1 6P6; dil.1:50, Bio Optica); NeuN (A60; dil.1:50, D.B.A., Milan, Italy); OLIG2 (polyclonal; dil.1:50; D.B.A.); Chromogranin A (DAC-A3; dil.1:100; DAKO); Synaptophysin (Polyclonal; dil. 1:100; DAKO); Neuron Specific Enolase (BBS-NC-6-H14; dil. 1:100; DAKO); alpha-fetoprotein (polyclonal; dil. 1:50; Bio Optica). Antigen retrieval was carried out with EDTA buffer and trypsin at pH6 prior to treatment for the following antibodies: GFAP, p53, EGFR, AE1/AE3, Neurofilaments, Ki67, OLIG2, Chromogranin A and Synaptophysin.

Results

The intraoperative smear demonstrated a hypercellular neoplasm, with a papillary pattern and frequent multibranched rigid structures (Fig. 1A,B). These structures were covered by cells showing dual epithelial and glial features, with elongated processes extending to the walls of thin blood vessels (Fig. 1C). In the smear, the neoplasm displayed a biphasic pattern. It was largely constituted by highly atypical, pleomorphic giant cells showing one or more hyperchromatic and large nuclei. Moreover, there were areas in which groups of smaller cells formed rare ependymal pseudorosettes (Fig. 2A,B). Small neoplastic cells were also scattered among the pleomorphic giant cells, similar in appearance to a swarm of bees (Figs. 1C, 2C). The following features were also observed: atypical mitoses; nuclear pseudoinclusions; cytoplasmic vacuoles; and eosinophilic, round, anucleated bodies (Figs. 1C, 4A,B). Cytological features overall led to the diagnosis of anaplastic ependymoma. The histological examination of the permanent sections showed (at scanning power) a biphasic pattern; large areas of papillary groups of sizeable anaplastic cells and areas of conventional ependymoma with smaller cells forming rare perivascular rosettes (Figs. 2B, 3C). There were also mitoses (2-3/1 mm^2) (Fig. 4C), geographic necrosis, and focal microvascular proliferation. Ki-67 labelling index was 15-20% (Fig. 3B). Diffuse immunopositivity was detected for GFAP (Fig. 3A) and S-100. There was little positivity for EMA, either as paranuclear dots or as bands of positive cell surface (Fig. 3C). Neuron specific enolase was positive in large areas (Fig. 4C). There was focal, perivascular positivity for cytokeratins. The other tested antibodies were negative. Round bodies and cytoplasmic vacuoles were also easily recognizable in the permanent sections (Fig. 4C).

A diagnosis of AGCE was rendered. After surgery,

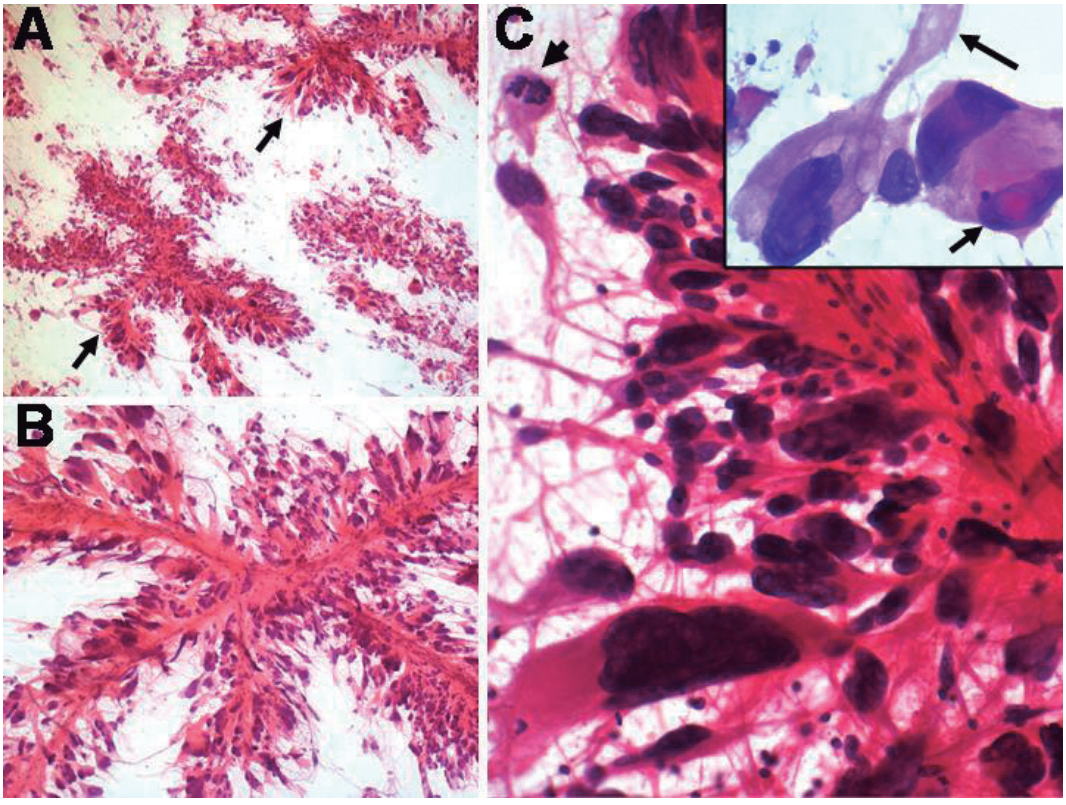

Fig. 1. Squash smear. Cohesive cells, with perivascular orientation, forming rigid structures, here appearing as starfish (**A**, arrows), with a central, thin vessel in each arm **(B)**. Tadpole-shaped neoplastic cells (**C**, the arrow head indicates a mitotic figure), with sharp borders and long cytoplasmic processes (**C** inset, long arrow); high grade nuclear pleomorphism and hyperchromasia are evident. A nuclear inclusion (C inset, short arrow). H & E. A, x 50; B, x 100; C, x 400

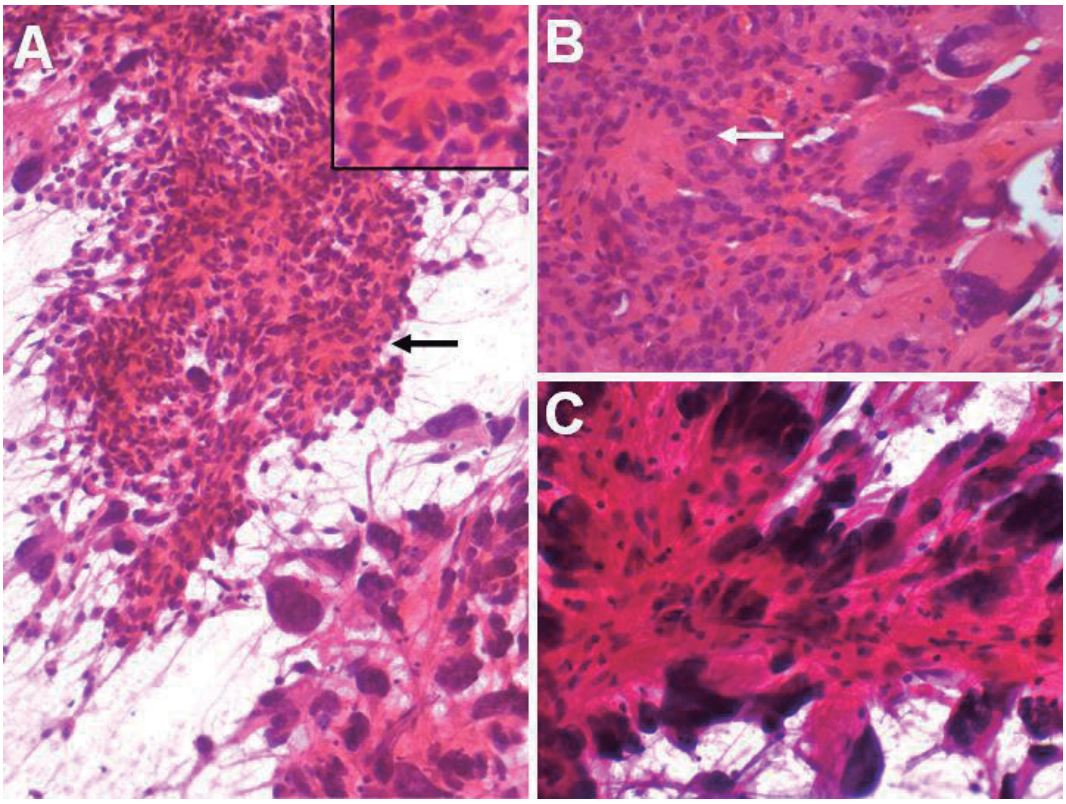

Fig. 2. Squash smear (**A, C**) versus permanent sections **(B).** In both, a dual pattern of the lesion is observable, with areas of conventional, cellular ependymoma constituted by small cells forming ependymal pseudorosettes (**A**, arrow and inset; **B**, arrow), opposite to areas with very large cells showing high grade nuclear atypia. Small neoplastic cells are also observable intermingled with the bigger ones **(C)**. H & E. A, x 100; B, x 200; C, x 400

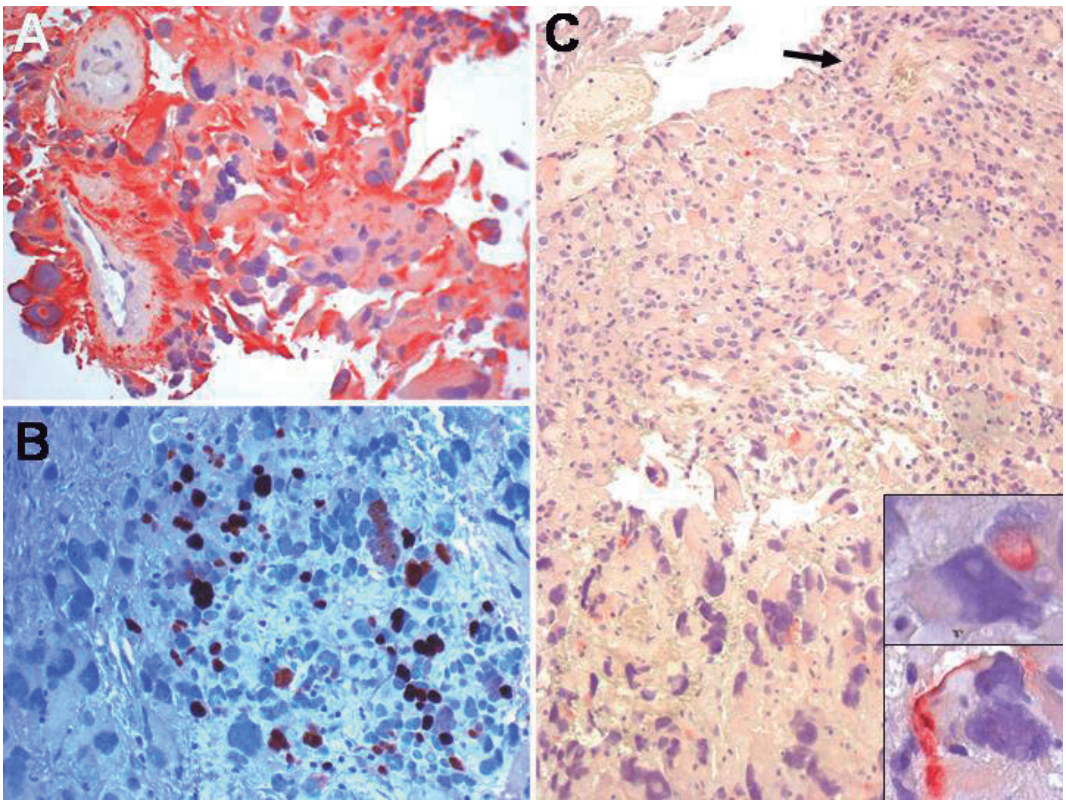

Fig. 3. Immunohistochemistry of permanent sections. Most tumor cells are strongly positive for GFAP **(A).** Ki-67 positivity is observable in tumor cells independently of their size **(B).** Dot-like, paranuclear (**C**, inset, upper part) or surface (**C**, inset, lower part) positivity for EMA; an ependymal pseudorosette (**C**, arrow) is observable in an area of conventional, anaplastic ependymoma. Immunohistochemistry. A, C: fuchsin counterstain; B: diaminobenzidine counterstain. A, x 100; B, x 200; C, x 100; C inset, x 400

radiation therapy was introduced. After a 3-year follow-up, the patient is well, with neither local recurrences nor metastatic spread.

Discussion

AGCE is an extremely rare subtype of ependymoma, only recently recognized as a distinct diagnostic entity. To our knowledge, it has yet to be described in the pineal region, which is, furthermore, a rare site for neoplasms (Al-Hussaini et al., 2009; Alexiou et al., 2012). Squash smear cytology is the preferred intraoperative technique in neurosurgical practice (Mitra et al., 2010).

Pleomorphic giant cells and/or anaplastic features can also be found in other low grade (e.g., subependymal giant cell astrocytoma [SEGA] and pleomorphic xanthoastrocytoma [PXA]) and high grade (e.g., anaplastic oligodendroglioma and giant cell glioblastoma) neoplasms (Martinez-Diaz et al., 2003; Karabagli et al., 2011; Koh et al., 2012; Li et al., 2012). In our case, the following features were critical clues to the intraoperative cytological diagnosis: papillary structures; giant cells with intranuclear inclusions; dual epithelial and glial properties of the tumor cells, and the presence of ependymal pseudorosettes. Furthermore, clinical presentation and additional cytological features are also useful in distinguishing AGCE from other lesions. SEGA, which arises from the lateral ventricles, is associated with the tuberous sclerosis syndrome. PXA is usually found in superficial sites and seldom presents in the pineal area (Srinivas et al., 2010; Thakar et al., 2012; Katayama et al., 2013). Moreover, lymphocytes and occasional lipidized cells are helpful cytological features of this lesion. In both SEGA and PXA, neoplastic cells are bizarre rather than anaplastic. In oligodendroglioma, the smear has a cloudy appearance, due to a sparse glial matrix with discohesive cells. Finally, in the smear, we did not observe either prominent microvascular vessel proliferation or relevant necrosis, which are usually observable in glioblastoma smears (Joseph, 2007).

Cytological features mirrored those observed in the permanent sections. Areas of conventional ependymoma were quite distinct from those showing anaplastic features, resulting in a biphasic pattern, as described in some of the GCEs reported in the literature, while in other reports there are giant cells scattered inside conventional ependymomatous areas (Karabagli et al., 2011; Koh et al., 2012; Li et al., 2012). In both the smear and the permanent sections, there were pseudorosettes and nuclear inclusions, hallmarks of ependymoma, as well as vacuoles, which have also been described in conventional ependymoma (McLendon et al., 2007). Likewise, eosinophilic globules, derived from cell cytoplasms, and the arrangement of anaplastic cells around blood vessels were evident in both the smear and in the permanent sections.

In GCE, immunopositivity is usually found for

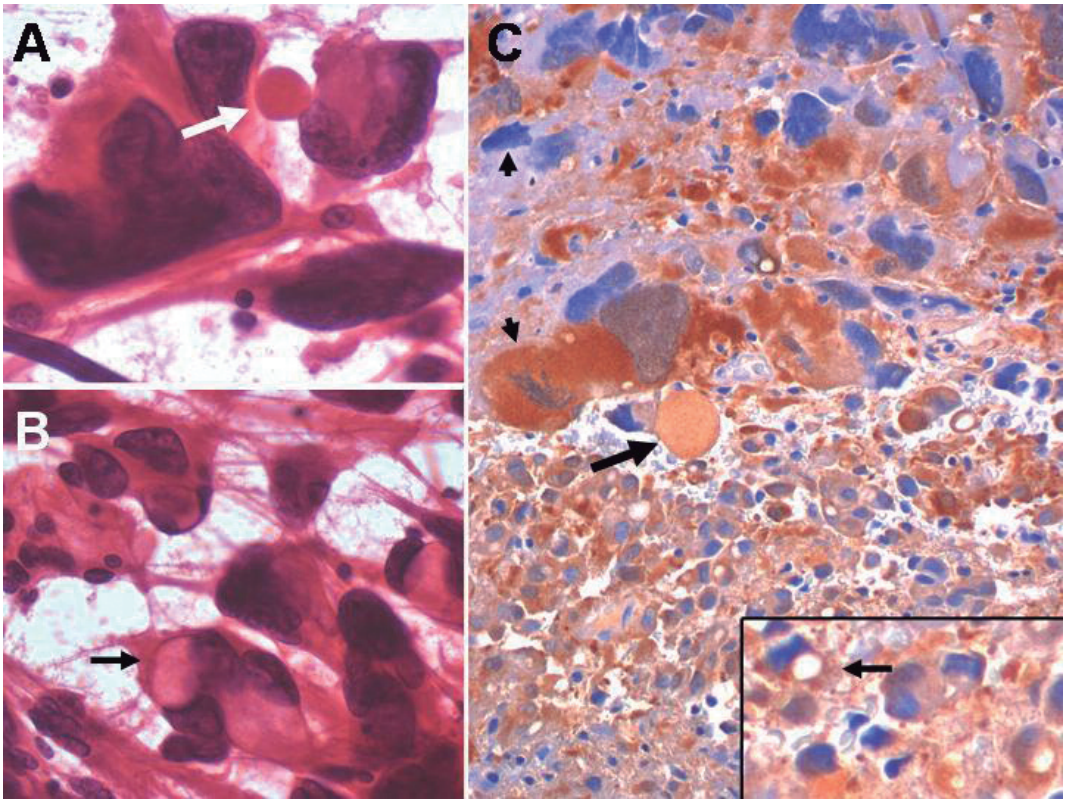

Fig. 4. Squash smear (**A, B**) versus permanent section (**C**). Round bodies (**A, C**, thick arrows), and cytoplasmic vacuoles (**A,** arrow; **C** inset, thin arrows). Diffuse immunopositivity for neuron specific enolase **(C)**, and mitoses (**C**, arrow heads) are observable. H & E (A, B); Immunohistochemistry (**C**, diaminobenzidine counterstain). x 400

GFAP, S-100 and EMA, as in our case. Neural markers and CD99 may also be positive (Andreiuolo et al., 2010; Koh et al., 2012). In our case NSE was positive, whereas other neural markers and CD99 were negative.

In the pineal region, in which only a few ependymomas have been described, other entities must be excluded. Germinoma, the most frequent neoplasm in this area (Al-Hussaini et al., 2009), as well as other germ cell tumors and pineal parenchymal tumors, can be excluded due to their morphologically and immunohistochemically different features.

There is a high risk of misdiagnosing AGCE as giant cell glioblastoma, which would need a different therapeutic approach due to its more aggressive behaviour. In fact, pineal glioblastoma has a high tendency of leptomeningeal and ependymal metastatic spread, and a survival rate not exceeding 1 year in children (Amini et al., 2006; Alexiou et al., 2012), whereas the 5-year survival rate for high grade ependymoma is 10-47% (Massimino et al., 2009; Martínez León et al. 2012). Therefore, it is important to provide a correct intraoperative diagnosis.

In the pineal region, GCE may be related to the pineal papillary tumor, which is supposedly of ependymal origin, and may also show pseudorosette formation around the vessels. However it shows quite a different pathological and immunohistochemical profile (Fèvre-Montange et al., 2006).

Anaplasia in GCE must be distinguished from degenerative atypia, which may occur in ependymomas. Palisading necrosis, microvascular proliferation and more than 10 mitoses per 10 HPF are thought to be the most reliable signs of anaplasia (Massimino et al., 2009). However, anaplasia in GCE does not always match with an aggressive course. Owing to its rarity, there is no consensus regarding GCE's histological grading system, which is, nonetheless, along with the extent of surgical resection, a primary determinant of survival (Fuller et al., 2010). The pathological diagnosis and grade are fundamental for subsequent therapeutic choices. In fact, there are no definitive radiological features that distinguish low from high grade ependymomas (Martinez León et al., 2012). In our patient, despite the presence of necrosis, mitoses and high grade atypia, the course was not aggressive, and neither local recurrences nor metastases were seen after 3 years. However, we are aware that a longer follow-up is necessary in order to draw reliable conclusions in this regard. Due to the extreme rarity of AGCE, reports of cases with cytological and histological comparative descriptions are extremely valuable. They will be helpful in making an intraoperative diagnosis, thus optimizing patients' treatment, and adding knowledge to the pathological and clinical characteristics as well as on the course of this rare entity.

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