Brain Tumor Pathol (2016) 33:19–26
DOI 10.1007/s10014-015-0237-1

ORIGINAL ARTICLE

Findings from frozen sections of spinal subependymomas: Is it possible to differentiate this diagnosis from other common spinal tumors?

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Received: 7 September 2015 / Accepted: 18 October 2015 / Published online: 29 October 2015

Abstract Subependymomas are slow-growing, benign neoplasms that are rarely found in the spinal cord. Because of the differences in the treatment plans, it might be very helpful for neurosurgeons to intraoperatively establish a diagnosis of spinal subependymoma, differentiated from other spinal intramedullary tumors. In this study, we analyzed frozen sections of spinal subependymomas to identify potential histological clues of spinal subependymomas to differentiate them from tumors that mimic spinal subependymoma. We reviewed the frozen sections and the corresponding permanent slides for 7 cases of spinal subependymoma. The spinal subependymomas showed several characteristic patterns, including, most importantly, an eccentric or both central and eccentric location in the axial plane. Histologically, they showed a (1) well-demarcated and multinodular mass with (2) low or moderate cellularity, (3) a microlobular pattern, and (4) small clusters of neoplastic cells. These features appear to be very specific to spinal subependymomas and could help differentiate them from ependymomas or astrocytomas. Although we might not be able to provide an exact diagnosis of all spinal subependymomas using these histological features, we hope that they help neuropathologists and neurosurgeons to adequately diagnose and treat spinal subependymomas.

Keywords Spinal cord · Subependymoma · Frozen section · Histology

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Introduction

Subependymomas are slow-growing, benign neoplasms that correspond histologically to the World Health Organization grade I. They are typically attached to a ventricular wall, where they are histologically characterized by clusters of isomorphic nuclei embedded in a dense fibrillary matrix of glial cell processes with frequent occurrence of small cysts, particularly in lesions originating in the lateral ventricles [1]. This type of neoplasm is rarely found in the spinal cord, and when it is, it occurs mainly as cervical or cervico-thoracic intramedullary lesions [2–4].

Of the spinal intramedullary neoplasms, ependymomas (including subependymomas) and astrocytomas are the most common. The treatment algorithm for spinal cord tumors varies based on the symptoms, location, and histology [5]. With intradural intramedullary tumors, the plan depends on whether the tumor is well-defined/circumscribed on magnetic resonance imaging. If it is well-defined/circumscribed, a maximum safe resection is attempted; if it is poorly defined/infiltrative, biopsy and adjuvant therapy should be considered [5]. The prognosis for spinal subependymomas is considered excellent [3, 5]. Gross total resection was previously attempted in 13 cases

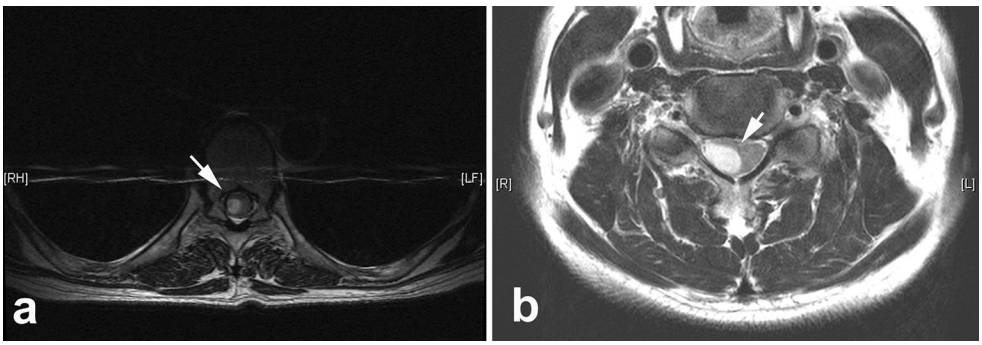

Fig. 1 Magnetic resonance imaging findings of Case 3 (**a**) and Case 5 (**b**). The T2 axial images show eccentrically located tumors (*white arrows*)

in a single institute when a frozen biopsy confirmed the diagnosis of subependymoma; spinal function was preserved, and there was neither progression nor recurrence of the residual tumors after a follow-up of at least 48 months [3]. Therefore, the authors suggested that revision operations are unnecessary.

Because of the differences in the treatment plans and to avoid unnecessary, aggressive treatment, it might be important to intraoperatively establish a diagnosis of spinal subependymomas, differentiated from other spinal intramedullary tumors, particularly astrocytomas or ependymomas [5]. However, it is practically impossible to reach all concordant diagnoses based on frozen sections, owing to potential limitations such as frozen section artifacts, a small amount of tissue, or lack of experience [6].

To the best of our knowledge, no in-depth evaluation of the features of frozen sections of spinal subependymomas has been conducted. Instead, because spinal subependymomas are very rare tumors, only sporadically reported cases and literature reviews are available [3, 4, 7–12], which primarily describe the characteristic histological features and indolent courses with good prognoses.

Therefore, in the present study, frozen sections of spinal subependymomas were analyzed, with an emphasis on differentiating spinal subependymomas from other common spinal intramedullary tumors, typical astrocytomas, and ependymomas. In addition, we summarized the histological clues in frozen sections that we believed could help to differentiate spinal subependymomas from other tumors that mimic spinal subependymomas.

Materials and methods

We retrieved samples of 13 cases of spinal intramedullary subependymomas, which were collected between 1998 and 2014, from the Pathology Department of the Yonsei University College of Medicine Severance and Gangnam Severance Hospital in Seoul, Korea. Among these cases, we collected the frozen section specimens that were available for 7 cases. These frozen tissue sections, 3–4 μm in thickness, were cut from surgical specimens using a cryostat for hematoxylin and eosin staining and analysis. All of the cases with frozen sections had corresponding permanent sections for the final diagnoses.

The histological findings of all of the original frozen sections were independently reviewed and analyzed for cellularity, architectural and histologic patterns, and nuclear features by two pathologists (Choi, SK and Kim, SH). The final diagnoses were based on both the frozen and permanent section tissues, which were submitted separately for this purpose. Pre-/post-operative neurologic function was assessed using the Modified McCormick classification [13–15]: Grade I, neurologically intact; Grade II, mild motor or sensory deficit; Grade III, moderate motor or sensory deficit; Grade IV, severe motor or sensory deficit; and Grade V, paraplegia or quadriplegia. The following clinical information was obtained from the medical records: age, sex, biopsy site, symptoms, and radiologic findings.

Results

There were 5 female and 2 male patients, with a mean age at diagnosis of 53.9 years (range, 31–69 years). The lesions were located in the cervical (3 cases) or thoracic (4 cases) spinal cord. Because the Case 1 patient had multiple lesions, she underwent 4 operations, in 2006, 2011, 2013, and 2014. A high-intensity signal was observed on T2-weighted imaging, and an iso- or low-intensity signal was observed on T1-weighted imaging in all cases. Except for Case 6, for which an axial image was not available, the tumors in all of the cases were eccentrically (Fig. 1) or both eccentrically and centrally located in the axial plane. No recurrence was observed, regardless of the extent of resection in all of the cases. The detailed clinical and radiologic findings are described in Table 1.

Table 1 Clinico-radiologic findings of the 7 cases with available frozen sections of spinal subependymoma

Case	Age (years)/sex	Symptoms	Tumor location	Tumor size (mm)	MRI T2	MRI T1	MRI T1 GD	Central vs. eccentric location	GTR vs. STR	Modified McCormick scale		Recurrence
1	44/F	Pain mainly in right lower extremity Tingling sensation in both lower extremities	C7–T5 (2006[a]) C6–T1 (2011[a]) C7–T5 (2013[a]) C6–T2 (2014[a])	12 × 14 × 75 11 × 11 × 24 13 × 9 × 22 5 × 7 × 8	High	Iso	Non-enhanced	Both Both Both Both	STR GTR GTR GTR	Pre-op Last follow-up	2 3	No
2	54/F	Pain mainly in left side of neck Tingling sensation in left lower extremities	C2–3	15 × 11 × 47	High	Iso and low	Slightly heterogeneous	Eccentric	GTR	Pre-op 1 month Post-op 18 months Post-op	1b 3 2	No
3	61/F	Pain and tingling sensation in both legs	T7–9	9 × 8 × 49	High	Iso and low	Slightly heterogeneous	Eccentric	GTR	Pre-op 6 months Post-op 60 months post-op	1b 3 2	No
4	54/F	Weakness and tingling sensation in right leg Urinary Sx	T10–L1	25 × 16 × 126	High	Iso	Slightly heterogeneous	Eccentric	STR	Pre-op 6 months Post-op 24 months Post-op	3 4 3	No
5	64/M	Tingling sensation and pain in right leg	C4–7	15 × 8 × 45	High	Iso	Slightly heterogeneous	Eccentric	STR	Lost to follow-up		No
6	69/F	Motor weakness in both legs	T6–9	64 (No axial)	High	Iso	Slightly heterogeneous	No axial	GTR	Pre-op 60 months Post-op	3 4	No
7	31/M	Pain mainly in right leg, paraparesis	T10–L1	23 × 9 × 80	High	Iso	Slightly heterogeneous	Eccentric	GTR	Pre-op 6 months Post-op 24 months Post-op	2 3 2	No

MRI magnetic resonance imaging, *urinary Sx* urinary symptoms, *Gd* gadolinium-enhanced, *GTR* gross total resection, *STR* sub-total resection

[a] Operative year

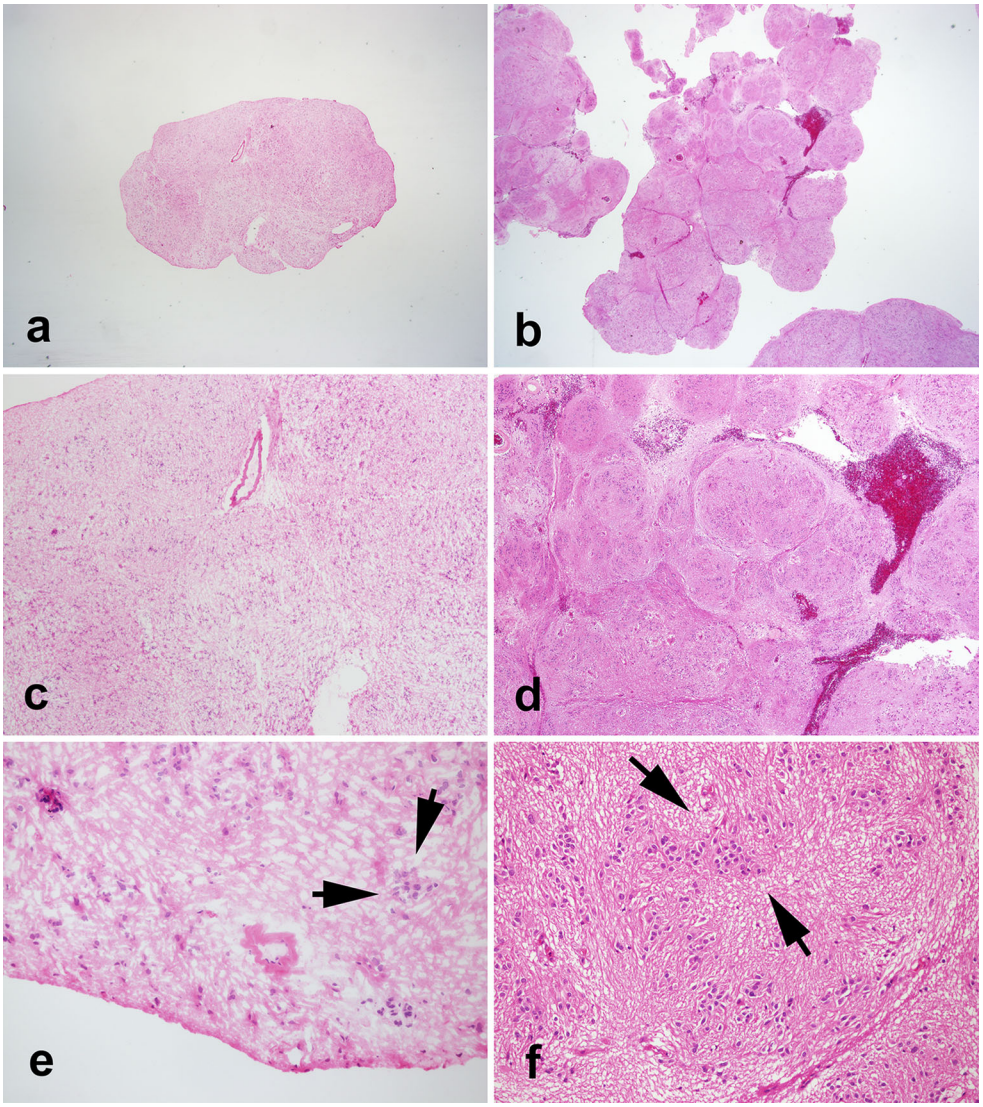

Fig. 2 Original frozen section slides and permanent slides of spinal subependymoma (Case 1) are shown in the *left* and *right rows*, respectively. The original frozen sections show a well-demarcated oval mass (**a**, ×12) with a vaguely microlobular pattern (**c**, ×40). In the high-power view, the tumor shows multiple small clusters of bland-looking tumor cells (*arrows*) with low cellularity (**e**, ×200). The corresponding permanent sections show a multinodular mass (**b**, ×12) with a prominent microlobular pattern (**d**, ×40) and small clusters of tumor cells (arrows) (**f**, ×200)

All cases showed low or moderate cellularity and bland-looking nuclei in the tumor cells, with the tumor cells clustering in the frozen section. In addition, macroscopically multinodular and microlobulated lesions at low power were important histologic findings in the frozen sections (Fig. 2). Interestingly, only the Case 4 patient showed microcystic changes that are frequently seen in brain subependymomas. The detailed histological findings of the 7 cases are described in Table 2.

In the intraoperative report, the tumor was described as "consistent with subependymoma" for 4 cases (Cases 1, 3, 4, and 6), "favoring ependymoma" (Fig. 3) for 2 cases (Cases 2 and 5), and "favoring pilocytic astrocytoma" for the remaining case (Case 7) because of the presence of Rosenthal fibers (Fig. 4) in the peritumoral gliotic area.

Discussion

Genomic information for spinal ependymomas, including subependymomas, has been a recent topic of active discussion [16, 17]. An examination of DNA methylation patterns in more than 500 samples of formalin-fixed paraffin-embedded ependymoma tissues showed that ependymomas could be classified in three major groups (spine, posterior fossa, and supratentorial) [16]. The spine

Table 2 Histologic findings of frozen sections from patients with spinal subependymoma

Case	Frozen sample size (cm)	Cellularity	Macroscopic pattern	Microscopic pattern	Fibrillar pattern	Cell clustering	Miscellaneous	Frozen Diagnosis
1	0.6 × 0.4	Low		Vaguely microlobulated	Densely packed	Small neoplastic cell clusters		c/w subependymoma
2	1.2 × 0.9	Moderate	Multinodular	Microlobulated	Long	Vaguely perivascular		Favoring ependymoma
3	0.7 × 0.3	Low	Vaguely multinodular	Microlobulated	Densely packed	Small neoplastic cell clusters		c/w subependymoma
4	0.5 × 0.5	Low		Vaguely microlobulated	Densely packed	Small neoplastic cell clusters	Mucoid microcytic changes	c/w subependymoma
5	0.6 × 0.4	Low	Multinodular		Densely packed	Vaguely perivascular		Defer, favoring ependymoma
6	1.0 × 0.5	Moderate	Multinodular	Microlobulated	Long	Small neoplastic cell clusters		c/w subependymoma
7	0.5 × 0.3	Low	Fragmented	Microlobulated	Long	No	Rosenthal fibers in the peritumoral area	Defer, favoring pilocytic astrocytoma

c/w consistent with

group included subependymoma, myxopapillary ependymoma, and (anaplastic) ependymoma subgroups. The spinal subependymoma subgroup had a characteristic 6q deletion and an excellent prognosis. Based on this information, a new therapeutic approach for these patients will be evaluated. Furthermore, these findings indicate that a correct histologic diagnosis will become more and more important.

Although the features of subependymomas in frozen sections are very similar to those of permanent sections, including low cellularity, a dense fibrillary matrix, and clusters of isomorphic nuclei, we identified several characteristic histological features in frozen sections that could serve as diagnostic clues.

Most importantly, spinal cord subependymomas were located eccentrically or both centrally and eccentrically in the axial plane. In comparison, spinal ependymomas are usually centrally located [3], and astrocytomas demonstrate fusiform growth [5]. Histologically, the first important characteristic was that the spinal cord subependymomas had well-demarcated and multinodular gross features. Excluding the fragmented specimen (Case 7), more than half (4/6) of the present cases had a well-demarcated and multinodular or vaguely multinodular appearance. Second, spinal subependymomas showed low or moderate cellularity. High cellularity would require differentiation from other tumors such as high-grade gliomas or ependymomas. Third, a microscopically microlobular pattern was observed in 6 of the 7 cases. Although this pattern can also be present in ependymomas, it could differentiate subependymomas from spinal astrocytomas. Finally, there were small clusters of neoplastic cells. Usually, the cluster consists of 15–20 tumor cells without a centrally located vessel; therefore, this feature appears to be very specific to spinal subependymomas and could help with differentiation from ependymomas (Fig. 2). A concordant diagnosis based on frozen specimens could have not been reached if the eccentric location and these four features were not prominent.

Because the histological features of spinal subependymomas and spinal ependymomas can overlap and present in similar combinations [1], spinal ependymomas represent the major differential diagnosis for spinal subependymomas in frozen sections. Spinal ependymomas can show a characteristic perivascular pseudorosette with long fibrillary processes; therefore, a vaguely perivascular pattern without small clusters of tumor cells in frozen sections (Cases 2 and 5) could lead to a misdiagnosis of ependymomas (Fig. 3). Upon further review of these two cases, we realized that we missed the vague multinodular pattern with small clusters of tumor cells in the original frozen slide. However, the characteristic histologic features of subependymoma were present in the permanent section slides for these cases.

In addition, the presence of long slender fibrillary processes with Rosenthal fibers in the peritumoral area can be confused with the features of pilocytic astrocytoma (Fig. 4) (Case 7).

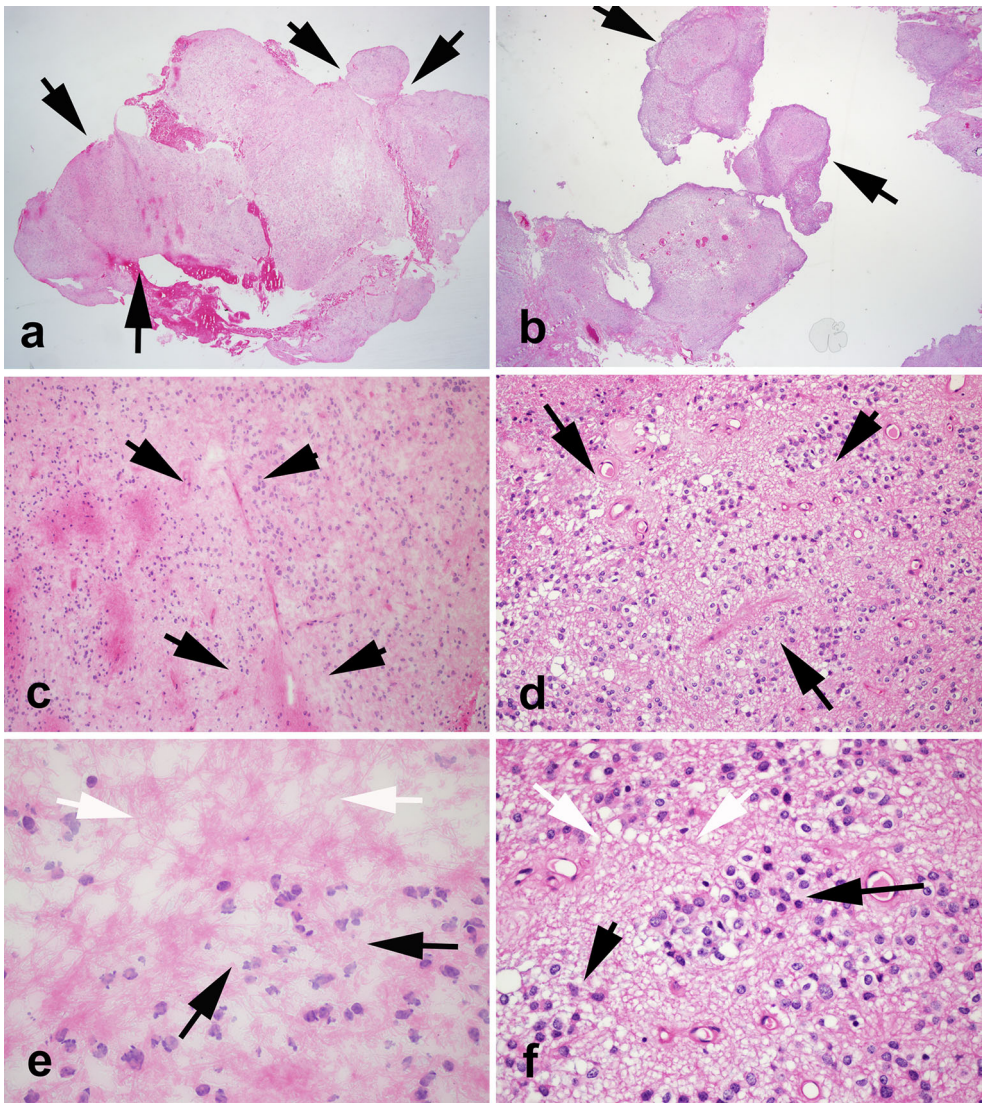

Fig. 3 Original frozen section slides and permanent slides of a spinal subependymoma (Case 2) that was intra-operatively described as "favoring ependymoma" are shown in the *left* and *right rows*, respectively. The original frozen sections show a multinodular (*arrows*), moderately cellular mass (**a**, ×12 and **c**, ×100) without a microlobulated pattern. Instead, ill-defined, perivascular, acellular areas (*arrows*) are noted (**c**, ×100). In the high-power view, a long fibrillary background is seen (*white arrows*) (**e**, ×400). The corresponding permanent sections show a multinodular (*arrows*) mass (**b**, ×12) with a vague perivascular pseudorosette-like pattern (*arrows*) (**d**, ×200). However, multiple small cell clusters are noted (*arrows*) (**f**, ×400). Upon review, a vague small cluster can be identified (*arrows*) (**e**, ×400), and the long fibrillary background is seen as fibrillary space between the small clusters of cell (*white arrows*) (**f**, 400)

Interestingly, the microcytic change [1] that is frequently seen in brain lesions rarely occurred in either the frozen or permanent sections of spinal subependymomas. Only one case (Case 4) had microcytic changes. Therefore, we suggest that this rare feature could result from the relatively smaller growth capacity in the spinal cord space than in the brain and ventricles.

The major limitations of the present study are the limited number of cases and diagnostic difficulty of small fragmented specimens, such as stereotactic biopsies. Despite the small sample, the present study is the first systematic review of a relatively large number of frozen sections of spinal subependymoma to be reported, to the best of our knowledge. The challenges with the diagnosis based on frozen sections of small fragmented samples, including stereotactic biopsy, seem to be relevant in all tumors or diseases and are not limited to spinal subependymomas. Therefore, we feel it is an important point that more than half of the cases showed a multinodular/microlobular pattern in frozen samples in actual clinical practice.

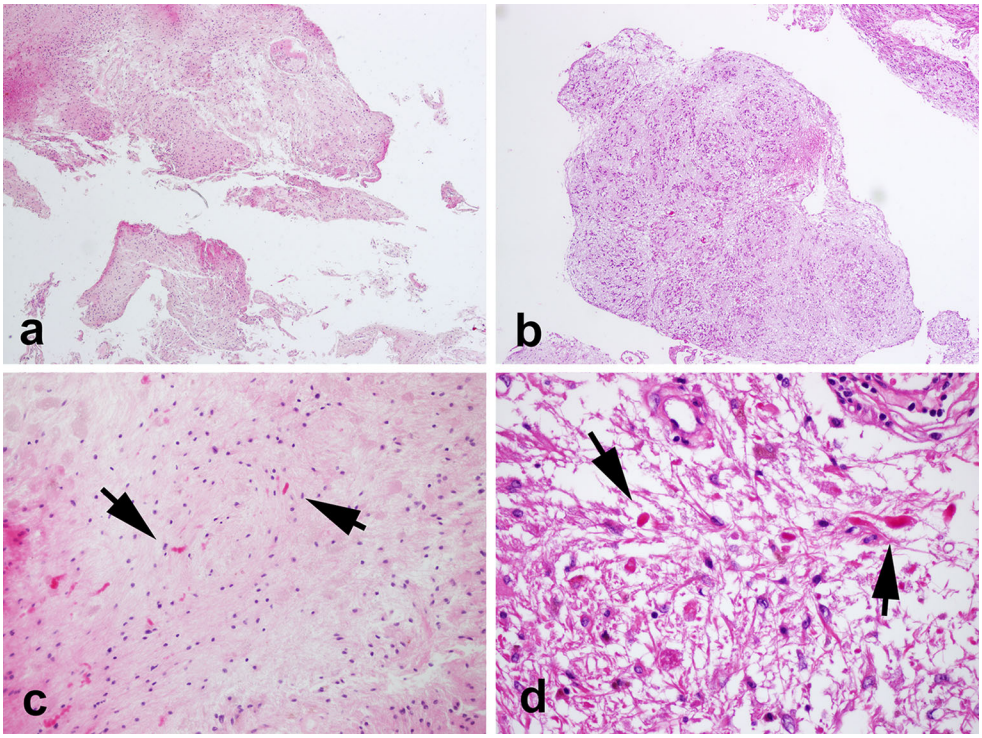

Fig. 4 Original frozen section slides and permanent slides of a spinal subependymoma (Case 7) that was intraoperatively described as "favoring pilocytic astrocytoma" are shown in the *left* and *right rows*, respectively. The original frozen sections show a fragmented, low-cellular mass (**a**, ×40) with several Rosenthal fibers (*arrows*) (**c**, ×200). The corresponding permanent sections show a multinodular mass with a microlobulated pattern (**b**, ×40). Rosenthal fibers (**arrows**) in the peritumoral area are noted (**d**, ×400)

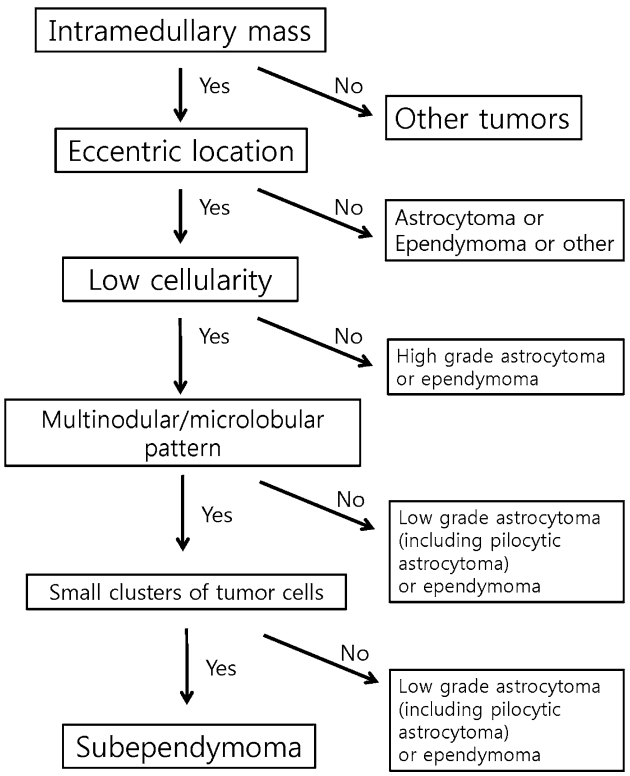

Fig. 5 Flow chart for the diagnosis of spinal subependymomas in frozen sections

Based on the clinical and histological features of the frozen sections described in this study, we created a flow chart with the aim of assisting with the adequate diagnosis and treatment of spinal subependymomas (Fig. 5) by neuropathologists and neurosurgeons.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

Funding This study was supported by a National Research Foundation of Korea (NRF) grant funded by the South Korean government's Ministry of Education, Science and Technology (MEST) 2013 (NRF-2013R1A1A2007357).

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