Retrospective Study on Accuracy of Intraoperative Frozen Section Biopsy in Spinal Tumors

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OBJECTIVE: **Histologic types and grades are critical in the diagnosis and treatment of spinal tumors. Intraoperative frozen section is a fast and easy method in confirming pathologic diagnosis during the operation. This study was undertaken to reveal the accuracy of intraoperative frozen section biopsy in order to make proper treatment plans.**

METHODS: **This retrospective study concerned patients who underwent spinal tumor surgeries from 1 January, 2012 to 31 December, 2016. Frozen section biopsy and permanent biopsy were compared, and cases that had differences were counted.**

RESULTS: **Thirty-seven cases out of 324 patients had discrepancies (11.4%). In discrepant cases 11 cases were ependymoma (29.7%) and 6 cases were schwannoma (16.2%). Among 34 patients who were finally diagnosed with an ependymoma, 11 cases had discrepancies, which was the highest mismatch rate among tumor final pathologic types. By frozen biopsy, astrocytoma ($n = 5$) and ependymoma ($n = 5$) turned out to be the most discrepant pathologic types and 16 frozen section biopsy cases were "null." Frozen biopsy astrocytoma ($n = 5$) mostly turned out to be ependymoma in 4 cases.**

CONCLUSIONS: **Pathologic findings from frozen biopsy for spinal cord tumors could not be corresponded to final diagnosis, especially when the results of frozen biopsy were ependymoma or astrocytoma. Therefore careful decision making for treatment plans is required.**

INTRODUCTION

Spinal tumors can cause serious neurologic morbidity and can be associated with mortality as well,[1] so their correct diagnosis and prompt treatment can be important. Although indications of central nervous system (CNS) tumor operations are based on the prognosis of the tumor and neurologic symptoms, in spinal surgeries functional outcomes are also greatly concerned.[2-4] Important predictors of functional outcomes include tumor histology, region of spinal cord tumor, age, and preoperative functional status.[2,4] Intraoperative total resections for all tumors might increase the rate of survival but may simultaneously cause loss of functional outcomes that could lead to loss of quality of life.[1,3,5] Thus treatment plan should be decided carefully to determine whether undergoing an operation or chemotherapy or radiotherapy might be beneficial.[1,4,6-8] If the patient is undergoing an operation, preoperative, intraoperative, postoperative decision making for treatment plans should be precise.

In establishing the differential diagnosis, location is an important feature. Magnetic resonance imaging is important in the imaging of spinal tumors and verification of the tumor location.[1,2,7-9] But histologic types and grades are also critical in the diagnosis and treatment of spinal tumors.[4,9] Frozen biopsy is a fast and relative easy intraoperative method to confirm pathologic diagnosis.[8,10-15] But if intraoperative frozen examination among some histologic types can turn out to be inaccurate, inadequate decision might be made during surgery and the extent of tumor resection may cause patients' functional outcomes to differ.[2-4] Surgery for spinal cord tumors is seen as not only a treatment modality but also a diagnostic modality.[12] Although there are many analyzed studies to find the accuracy of intraoperative frozen examination among other tumors,[8,12,15] only a few studies have analyzed spinal tumors. This study is to reveal the accuracy of intraoperative frozen examination among numerous histologic

Key words
- Astrocytoma
- Ependymoma
- Frozen biopsy
- Intramedullary tumor
- Spinal cord
- Spinal tumor

Abbreviations and Acronyms
CNS: Central nervous system
MRI: Magnetic resonance imaging

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types of spinal cord tumors to assist the neurosurgeon's intraoperative decision making for the extent of tumor removal.

METHODS

This study is a single-institute retrospective study. We overviewed cases of spinal tumor surgeries during a 5-year period ranging from 1 January, 2012 to 31 December, 2016 at the Yonsei University Gangnam Severance Hospital. Patients who have had spinal cord tumor removal surgeries according to the medical operation records were included in the criteria. All patients included in the criteria had undergone intraoperative frozen examination and permanent final pathologic diagnosis. Patients without intraoperative frozen examination were excluded from the criteria. Frozen section and permanent section analyses were compared. All pathologic procedures including intraoperative frozen consultation and permanent section analyses were conducted by our department of pathology, initially by subspecialized neuropathologists with a following review of a well-experienced expert. Pathologic diagnosis was based on World Health Organization grade. For statistical conveniences, subependymoma was considered as ependymoma, and anaplastic meningioma and meningothelial meningioma were considered as meningioma. We defined uncertain and nondiagnostic pathologic readings, such as "defer to permanent," "see permanent biopsy for pathologic confirm," "tumor A versus B," "presence of tumor," inappropriate specimens "normal tissue," and "crushed, squeezed," as *null*. For readings such as "tumor A is most likely, B less likely" the readings were concerned to be tumor A. The Fisher exact test was used to analyze which histologic type is statistically significant. All statistical tests were conducted using IBM SPSS, version 23.0 (Armonk, New York, USA).

RESULTS

Pathologic Type of Spinal Tumors

A total of 324 patients underwent frozen biopsy during spinal tumor surgeries through our study date. The most frequent final pathologic type was schwannoma ($n = 147$), and the following were meningioma ($n = 47$), ependymoma ($n = 34$), astrocytoma ($n = 6$), and other miscellaneous tumors ($n = 90$).

Thirty-seven (11.4%) out of 324 patients exhibited discrepancy between frozen section biopsy and final pathologic diagnosis. In other words, the accuracy of intraoperative frozen examination in our study was 88.6%. Eleven cases of mismatch were pathologically confirmed as ependymoma on final diagnosis, and 6 cases of mismatch as schwannoma (Table 1). One case was not diagnosed; it was mentioned earlier as "null." A total of 34 cases were pathologically confirmed as ependymoma, which means that 11 out of 34 ependymoma patients had inaccurate intraoperative frozen examination results. Among 324 patients, 147 of them were confirmed as schwannoma and 6 patients had inaccurate frozen examination results.

Ependymoma and Astrocytoma the Most Discrepant Histologic Types

As seen from the frozen biopsy, the pathologic types of ependymoma and astrocytoma were the most frequent type of tumor, which caused discrepancy ($P < 0.05$), while schwannoma ($P = 0.201$) and meningioma ($P = 0.271$) were statistically insignificant ($P > 0.05$) and other tumors were statistically insignificant because of the limited amount of cases (Table 2). Five patients who were diagnosed with an ependymoma in the frozen section turned out to be different in the final diagnosis, and the other 5

Table 1. Permanent Pathologic Diagnosis of Discrepant Cases

Histologic Type of Tumor	Number of Cases (%)
Angiolipoma	1 (2.7%)
Astrocytoma	1 (2.7%)
Cavernous hemangioma	2 (5.4%)
Chondrosarcoma	1 (2.7%)
Diffuse midline glioma	1 (2.7%)
Ependymoma	11 (29.7%)
Epithelioid tumor	1 (2.7%)
Hemangioblastoma	1 (2.7%)
Nontumor	2 (5.4%)
Lipoma	1 (2.7%)
Malignant peripheral nerve sheath tumor	1 (2.7%)
Meningioma	2 (5.4%)
Metastasis	1 (2.7%)
Myxoma	2 (5.4%)
Paraganglioma	2 (5.4%)
Schwannoma	6 (16.2%)
Null	1 (2.7%)
Total	37 (100%)

Table 2. Frozen Biopsy of Discrepant Cases

Histologic Type of Tumor	Number of Cases (%)
Astrocytoma	5 (13.5%)
Chondroma	1 (2.7%)
Chordoma	2 (5.4%)
Ependymoma	5 (13.5%)
Hemangioma	1 (2.7%)
Inflammation	1 (2.7%)
Meningioma	1 (2.7%)
Metastasis	1 (2.6%)
Neurogenic tumor	1 (2.7%)
Null	16 (43.2%)
Schwannoma	1 (2.7%)
Spindle cell tumor	2 (5.4%)
Total	37 (100%)

Table 3. Cases of Frozen Diagnosis of Ependymoma and Astrocytoma

Case Number	Age (years)/Sex	Frozen Biopsy	Preoperative Radiologic Dx	Permanent Diagnosis
1	45/Female	Ependymoma	Lumbar/IM/Ependymoma	Schwannoma S-100: diffuse strong positive GFAP: focal positive EMA and synaptophysin: negative
2	30/Male	Ependymoma	Thoracic/Both intradural and extradural components/Neurogenic tumor	Malignant peripheral nerve sheath tumor
3	57/Female	Ependymoma	Sacral/IDEM/Myxopapillary ependymoma seeding	Paraganglioma CD99 (MIC2): strong positive Chromogranin A: positive S-100: loss of sustentacular cells EMA and CK (AE1/3) and GFAP: negative
4	37/Male	Ependymoma	Lumbar/IDEM/Schwannoma	Paraganglioma, synaptophysin, and NSE: positive Chromogranin: dotlike positive S-100: presence of sustentacular cells Ki-67: 2∼3%
5	33/Female	Ependymoma	Cervical/IM/Ependymoma	Diffuse midline glioma H3 K27M-mutant
6	30/Female	Astrocytoma	Cervical/IM/Astrocytoma	Multifocal perivascular lymphocytic infiltrations with focal macrophage
7	36/Female	Astrocytoma	Thoracic/IM/Astrocytoma	Ependymoma GFAP and CD99 (MIC2): positive EMA: negative Ki-67: <5%
8	31/Male	Astrocytoma	Thoracic/IM/Ependymoma	Subependymoma
9	44/Female	Astrocytoma	Cervical/IM/N/A (noncontrast image)	Ependymoma GFAP, S100: positive EMA: focal positive Ki-67: <1%
10	17/Female	Astrocytoma	Cervicothoracic/IM/Diffuse astrocytoma	Subependymoma GFAP: positive IDH1: negative EMA: positive Galectin3: negative Ki-67: 1%

Dx, diagnosis; GFAP, glial fibrillary acidic protein; IM, intramedullary; EMA, epithelial membrane antigen; IDEM, intradural-extramedullary; CK, creatine kinase; NSE, neuron-specific enolase.

diagnosed as astrocytoma were ultimately mismatched (Table 3). Most cases that were originally thought to have astrocytoma on frozen biopsy turned out to have ependymoma ($n = 4$) on permanent analysis (Figure 1).

Frozen Biopsy—"Null"

Sixteen cases were considered nondiagnostic "null" due to the uncertain frozen section diagnosis mentioned earlier. Four patients turned out to have ependymomas in the final pathologic diagnosis, followed by 3 patients with schwannomas and 2 patients with myxomas (Table 4). "Null" didn't depend on age, sex, pathologic type, or anatomic location (extradural/intradural-extramedullary/intramedullary). "Null" consisted 43.2% of all discrepancy cases, which means the limitation of the frozen section itself could be a cause of discrepancy.

DISCUSSION

Spinal tumors are rare but can cause significant morbidity in terms of limb dysfunction and can be associated with mortality as well.[1] Their correct diagnosis and prompt treatment can be important. In establishing the differential diagnosis for a spinal lesion, location is the most important feature.[1,6,13] With the help of magnetic resonance imaging (MRI), which plays a key role in the imaging of spinal tumors, spinal tumors are classified as extradural, intradural-extramedullary, or intramedullary.[1,3,5,6,15] In general, extradural spinal lesions are the most common location (60% of all spinal tumors), with the majority of lesions originating from the vertebrae.[1] The most frequent extradural tumors are metastatic tumors, while primary bone tumors are more uncommon.[1] Intradural tumors are rare, with an annual incidence of approximately 2–4 tumors per 100,000 people, and

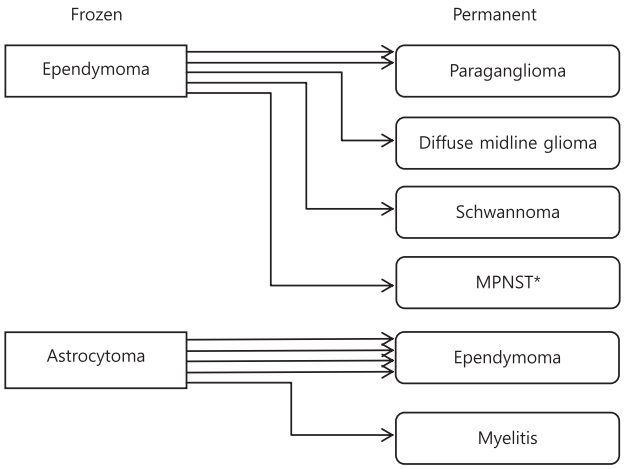

Figure 1. Flow chart of discrepant cases from frozen biopsy. Each *arrow* represents a case. Ependymoma and astrocytoma were the most frequent discrepant histologic type. MPNST, malignant peripheral nerve sheath tumor.

present with neurologic symptoms or deficits.[1] The majority of intradural tumors are intradural-extramedullary tumors, which are approximately 30% of all spinal tumors. They consist of meningiomas, nerve sheath tumors such as schwannomas and neurofibromas, and distant metastases.[1] Intramedullary tumors are uncommon, consisting of only 10% of all spinal tumors.[1] Astrocytoma and ependymoma comprise the majority of intramedullary tumors, with ependymoma occurring at double the frequency of astrocytoma.[1,2,4,7,9,13,16,17] It is widely known that astrocytoma is more aggressive than ependymoma.[1,2,4,7,9,13,16,17] Clinical progression in astrocytoma is much more rapid than in ependymoma, and often there is only a short interval between the onset of neurologic symptoms and permanent disability or death.[1,2,4,7,9,13,16,17]

In spinal tumors surgical resection is the primary diagnostic and treatment modality.[1-6,9,17] Surgical resection allows us to obtain pathologic diagnosis and also allows debulking or full resection of tumor in order to decompress the neural structures that might have caused neurologic symptoms. Furthermore, pathologic diagnosis of spinal tumor is one of the most significant predictors for good prognosis.[6,7,9] For example, lower grades have better prognosis and astrocytoma has a worse prognosis than ependymoma. Astrocytomas are infiltrative and may have an irregular margin from the normal spinal cord.[9] This may not be markedly visible on preoperative MRI or grossly apparent during the operation.[16] There is a trend that the extent of resection of low-grade astrocytomas correlates with better outcome.[4,9] As a result, the risk of subtotal resection must be balanced against the risk of neurologic impairment regarding the prognosis.[2,16] Another important prognostic indicator is the patient's condition at the time of surgery. Previous studies suggest that the better the patient's preoperative clinical status is, the better the postoperative outcome is.[2,16] Thus preoperative functional status also needs to be considered before spinal tumor surgeries (for spinal tumors, the modified McCormick Scale is considered the standard outcome tool).[2,4,5,17] The decision to proceed to surgery in patients with a slowly progressive, minor motor or sensory deficit is difficult, particularly if imaging studies suggest the presence of an infiltrating astrocytoma, which may not be removed without significant risk of neurologic deficit.[5,7] Therefore we should carefully plan before the operation, considering the multidimensional factors.

If histologic grades are important, frozen biopsy, a fast and relatively easy intraoperative method to confirm pathologic diagnosis, is also important. But if frozen biopsy among some histologic types can turn out to be inaccurate, inadequate decision might be made during surgery and the variant extent of tumor resection may cause the patient's functional outcomes to differ.

Although previous studies have analyzed the accuracy of frozen biopsies for other tumors, studies that have focused on spinal tumors are scarce. In a previous study about accuracy of frozen biopsy in CNS tumors, Geramizadeh et al[8] in 2010 compared the overall accuracy of frozen section analysis with permanent section analysis and reported that CNS lesions had the highest rate of discrepancy.[8] The accuracy of intraoperative frozen examination for intracranial lesions has ranged from 69%–98.5% in previous studies.[8,11,15]

This study differs from previous studies in that it is focused mainly on spinal tumors but not all CNS tumors because the relative proportions of tumor types found as primary tumors in the spinal cord differ from those reported in the brain.[8] Also, the distinctive symptoms of spinal cord lesions are different from other CNS symptoms. Recently there was a similar study of accuracy of frozen biopsy among 67 spinal tumors by Kobayashi et al[15] in 2018 and rated 86.6% with intramedullary lesions having significantly lower accuracy.

In our study, we had an accuracy of 88.6% among 324 cases. Intramedullary tumors, such as ependymoma or astrocytoma, were the most discrepant pathologic types, concordant with the previous study. However, according to our knowledge, this is the second study about the accuracy of intraoperative frozen examination in spinal tumors with large cases and because these 2 studies are from single institutes, further studies among other institutes might be necessary.

Many factors can cause discrepancies in frozen biopsy. Sampling error such as poor resection, poor selection, degeneration, or necrosis is a main cause, especially for cases of intramedullary spinal cord lesions in which an adequate amount of sampling might be difficult in order to avoid damage to normal neural structures that might cause neurologic symptoms. Compared with permanent section, the frozen biopsy method itself has limitations, such as freezing artifacts, xylene artifacts, poor-quality section, and bloated cell morphology.[8,11,15] Moreover, limited time and information about the patient might lead to inappropriate reading by the pathologist.[8,11,15]

We assumed that a pathologic type of tumor can also act as a factor causing discrepancy, and according to our study ependymoma and astrocytoma were the most discrepant histologic type. Because ependymoma and astrocytoma are intramedullary lesions, sampling volume is relatively small and might be the reason for inaccuracy in intraoperative frozen examination.

Table 4. Cases of Frozen Diagnosis of "Null"

Case Number	Age (years)/Sex	Frozen Biopsy	Preoperative Imaging	Permanent Diagnosis
1	58/Male	Null	IM	Ependymoma
2	72/Male	Null	ED/Metastasis	Metastatic adenocarcinoma
3	19/Female	Null	Leptomeningeal seeding/Ependymoma	Ependymoma GFAP: positive EMA: positive, perinuclear dot pattern Synaptophysin: negative Ki-67: 5%
4	39/Female	Null	Leptomeningeal seeding/N/A (enhancing lesion)	Hemangioblastoma S-100 & EMA: positive SMA & CD31: positive in vessel GFAP: negative
5	54/Female	Null	N/A (no interpretation)	Nerve sheath myxoma S100: positive GFAP, EMA: negative Ki-67: 10%
6	45/Female	Null	IM/astrocytoma	Astrocytoma
7	64/Male	Null	IDEM Intradural neurogenic tumor	Nerve sheath myxoma
8	42/Male	Null	ED/Hemangioma	Inflammation IgG4-related meningeal disease
9	43/Male	Null	IDEM/Meningioma	Schwannoma
10	51/Female	Null	IM Conus medullaris lipoma	Lipoma
11	43/Male	Null	IM/cavernous malformation	Cavernous hemangioma
12	57/Female	Null	IM/ependymoma	Atypical meningioma EMA & vimentin: positive S-100 & GFAP: negative Ki-67: 5%
13	46/Male	Null	IDEM/Ependymoma	Ependymoma GFAP: positive EMA: dotlike pattern, focal
14	61/Male	Null	IM/Tumor with cord edema	Ependymoma OLIG-2: negative EMA: focal positive GFAP: positive Ki-67 3%
15	62/Male	Null	N/A (no interpretation)	Schwannoma S-100: positive GFAP: negative
16	57/Female	Null	IDEM/Meningioma	Schwannoma

IM, intramedullary; ED, extradural; IDEM, intradural-extramedullary; GFAP, glial fibrillary acidic protein.

What we defined as "null" is a concept of error or nondiagnostic results in intraoperative frozen examination and is also associated with sampling error, poor resection, or the limitations of frozen biopsy, such as the artifacts mentioned earlier.

From the discussion of our study with the pathologic department, there was an opinion from the pathologist's view of intraoperative frozen examination that there may be insufficient information about the patient and most of the "null" or nondiagnostic results come from inadequate targeting of the lesion, uncertain histologic grade, and inadequate sample, such as insufficient volume of sample, fat, or calcification in the sample.

For statistical convenience we considered ependymoma as a group, but because subependymoma and ependymoma are different in World Health Organization grades and also for astrocytoma, which is our main concern, there was consideration of unclearly delineated subgroups in our study. Fortunately, from our

review there was no subependymoma or other ependymoma or astrocytoma subgroups in our intraoperative frozen examination. This was probably because of the difficulty in determining the histologic grade during frozen examination. There were 2 subependymomas in our pathologic diagnosis. **Table 3** describes other subgroups.

We can improve the accuracy of frozen biopsy in several ways. For example, the use of additional methods such as intraoperative cytology might be beneficial. In a previous study, squash preparation, a method of cytology, was shown to improve frozen section accuracy in CNS tumors.[12] Also, dynamic and active communication among surgeons and pathologists can give plenty of clinical information about the patient, thus reducing the discrepancy rate.[8,11,15] Telepathology technique among pathologists is also a rising technique in remote areas.[8,11,15] Furthermore, careful preoperative reviewing of images with radiologists might reduce inaccuracy of frozen biopsy in terms of giving sufficient clinical information to the pathology.[8,11,15] In our study, due to patients who had preoperative image work-up in other institutes and brought only the image file, several cases lacked interpretation of preoperative magnetic resonance readings by radiologists. These cases proved to be statistically challenging due to the missing data, but if we identify and disregard these cases with interrupted records, MRI might be a factor helping to reduce the inaccuracy of intraoperative frozen examination.

Because this study was retrospective, studies are subject to significant selection and information biases, which would be our limitation in this study.

CONCLUSION

Astrocytoma and ependymoma were the most discrepant pathologic type of spinal tumors in intraoperative frozen examination. Therefore in treating spinal tumors in which intraoperative frozen examination was confirmed as ependymoma or astrocytoma, in other words intramedullary tumor, careful decision making for treatment plans is required and further methods including imaging methods, pathologic methods such as smearing, and active communication with the pathologist must be considered preoperatively and intraoperatively for assistance. And the extent of excision should be considered carefully, especially in intramedullary tumors, concerning neurologic outcomes and prognosis.

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