

Molecular Features and Prognostic Factors of Pleomorphic Xanthoastrocytoma: A Collaborative Investigation of the Tohoku Brain Tumor Study Group

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Abstract

Pleomorphic xanthoastrocytoma (PXA) is a rare glial tumor, however, its histological differentiation from high-grade gliomas is often difficult. Molecular characteristics may contribute to a better diagnostic discrimination. Prognostic factors of PXA are also important but few relevant reports have been published. This study investigated the molecular features and prognostic factors of PXAs. Seven university hospitals participated in this study by providing retrospective clinical data and tumor samples of PXA cases between 1993 and 2014. Tumor samples were analyzed for immunohistochemical (IHC) neuronal and glial markers along with Ki67. The status of the *BRAF* and *TERT* promoter (*TERTp*) mutation was also evaluated using the same samples, followed by feature extraction of PXA and survival analyses. In all, 19 primary cases (17 PXA and 2 anaplastic PXA) were included. IHC examination revealed the stable staining of nestin and the close association of synaptophysin to NFP. Of the PXA cases, 57% had the *BRAF* mutation and only 7% had the *TERTp* mutation. On univariate analysis, age (≥ 60 years), preoperative Karnofsky performance status

Received May 12, 2020, Accepted July 1, 2020

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(KPS) ($\leq 80\%$), and marked peritumoral edema were significantly associated with progression-free survival (PFS). No independent factor was indicated by the multivariate analysis. In conclusion, PXA was characterized by positive nestin staining and a few *TERT* mutations. The neuronal differential marker and *BRAF* status may help in diagnosis. Patient age, preoperative KPS, and marked perifocal edema were associated with PFS. The present study is limited because of small number of cases and its retrospective nature. Further clinical study is needed.

Keywords: differential diagnosis, neuronal differentiation, pleomorphic xanthoastrocytoma, prognostic factors, TERT promoter mutation

Introduction

Pleomorphic xanthoastrocytoma (PXA) is a rare glial tumor that comprises $<1\%$ of all astrocytic neoplasms and is mostly observed in children and young adults.¹⁾ PXAs and glioblastomas (GBMs) share a pleomorphic histological appearance, resulting in difficulties for differential diagnoses.^{2,3)} However, most PXAs show better prognosis and are classified as WHO grade II, moreover, PXA may be cured with a complete resection without additional radiochemotherapy.⁴⁾ Thus, methods of definitive diagnosis using preoperative imaging, and intraoperative rapid and postoperative histological examinations are desired. Several neuronal markers and *BRAF* mutation are reported to be characteristic of PXAs^{1,5,6)}, however, results are not yet conclusive. Better discrimination using other molecular features would help to properly diagnose and treat patients with PXAs.

Some PXAs show malignant histological features and aggressive clinical behavior. *BRAF* mutation may relate to better prognosis^{4,7)}, however, no reliable prognostic factor has yet been established. Considering treatment, the roles of radiotherapy and chemotherapy for PXAs have also not been well defined.

Therefore, the current study investigated molecular features and prognostic factors of PXAs.

Materials and Methods

Study design

A retrospective, observational multicenter study by the Tohoku Brain Tumor Study Group including Akita, Hirosaki, Yamagata, Tohoku, and Niigata Universities, together with Iwate and Fukushima Medical Universities. This study was approved by the ethics committee of the above institutions with the approval numbers of 1286, 2016-1047, H27-287, 2015-1-718, 1488, H24-96, 2065, respectively.

Clinical data and human tumor specimens

Clinical data and tissue samples of a total of 23 primary cases that were initially diagnosed as PXA

between 1993 and 2014 in each institute were provided for this study. Clinical information included patient's age, sex, preoperative Karnofsky performance status (KPS), extent of resection, radiotherapy, chemotherapy, progression-free survival (PFS) and overall survival (OS) as listed in Table 1.

Tumor samples from all cases were centrally reviewed by expert neuropathologists (Yoichi Nakazato, Hidaka Hospital, and Sumihito Nobusawa, Gunma University) prior to further analysis in this study. Only cases diagnosed as PXA based on the previous version of the WHO Classification of Tumors of the Central Nervous System (WHO2007) were included in the present study.

Radiological findings on magnetic resonance imaging

Tumor location and size, together with peritumoral edema were evaluated on a preoperative magnetic resonance imaging (MRI). The extent of resection was qualitatively classified on a postoperative MRI.

Histology and immunohistochemistry

Adjacent 4- μm -thick sections served for hematoxylin & eosin (HE) and reticulin-staining, and immunohistochemical (IHC) staining, respectively. IHC staining was performed with the Ventana Benchmark-XT (Roche Diagnostics K.K. Tokyo, Japan) targeting at GFAP (1:5000),⁸⁾ S-100 (1:10000),⁸⁾ nestin (1:100, Millipore), synaptophysin (1:1, Roche), NFP (1:100, Invitrogen), NeuN (1:2000, Millipore), CD34 (1:1, Roche), IDH1 R132H (1:50, Dianova), and Ki67 (1:1, Roche). For Ki67 analysis, tumor areas with the highest Ki67 labeling were selected, and the images were captured with a microscopic digital camera, then, the labeling index was calculated using the Image-J software.⁹⁾

DNA extraction and direct DNA sequencing for *BRAF* and *TERT* promoter mutations

DNA was extracted from FFPE tissue sections, as previously described,^{10,11)} and was amplified and sequenced using the primers described previously.^{12,13)} The polymerase chain reaction (PCR) products were

Table 1 Clinical and radiological characteristics, treatment modalities, and outcomes of patients with PXAs

Case	Sex	Age	Initial symptoms	Pre-op KPS	Location	Size (cm)	Peritumoral edema	Extent of resection	RT (dose, fr)	ChT	Central review	Rec (mo)	F/U (mo)	Outcome
1	F	7	NF1	100	Rt. frontal	4.0	None	TR			PXA		158	Alive
2	F	13	Sensory disorder	100	Rt. insula	4.0	Moderate	STR	SRT (40 Gy, 9 fr)		PXA	113	165	Alive
3	F	60	Motor aphasia, visual field constriction	90	Lt. temporal	7.7	Moderate	STR			PXA	12	72	Alive
4	M	30	Sensory disorder	100	Lt. parietal	7.0	Moderate	TR			PXA		59	Alive
5	M	65	Gait disorder	50	Lt. temporal	3.5	Marked	TR	Local RT (60 Gy, 30 fr)	ACNU IFN TMZ	PXA**	7	28	Died
6	F	59	Headache, hemiparesis (intratumoral hemorrhage)	10	Lt. temporal	2.0	None	TR			PXA	5	38	Died
7	M	18	Seizure	90	Lt. temporal	3.5	Marked	STR			PXA	37	96	Alive
8	F	20	Seizure	90	Lt. parietal	2.3	Moderate	TR			PXA		42	Alive
9	F	58	Seizure	90	Rt. frontal	5.5	Moderate	TR			PXA		204	Alive
10	M	20	Seizure	90	Rt. temporal	2.0	Moderate	TR			PXA		61	Alive
11	F	68	Sensory aphasia	80	Lt. parietal	6.0	Marked	STR	Local RT (60 Gy, 30 fr)		PXA	6	95	Alive
12	M	16	Seizure	100	Lt. temporal	4.0	Moderate	STR	Local RT (50 Gy, 25 fr)		PXA		321	Alive
13	F	11	Seizure	60	Rt. temporal	6.0	Moderate	PR		PCV	PXA		91	Alive
14	M	36	Headache, nausea	90	4th ventricle	2.5	None	TR			PXA		120	Alive
15	M	18	Seizure	100	Rt. temporal	4.5	Marked	TR			PXA	47	89	Alive
16	M	17	Seizure	100	Rt. parietal	2.5	Moderate	TR			PXA		79	Alive
17	F	44	Seizure	100	Rt. temporal	8.0	None	TR			PXA		45	Alive
18	F	16	Headache, nausea, sensory disorder	90	Lt. parietal	7.0	Moderate	STR	Local RT (60 Gy, 30 fr) Boost SRT (20 Gy, 5 fr)	TMZ	A-PXA		77	Alive
19	M	74	Seizure	70	Lt. frontal	3.5	Moderate	STR	Local RT (60 Gy, 30 fr)	TMZ	A-PXA	4	9	Died

*The longest diameter determined on gadolinium-enhanced T1-weighted magnetic resonance imaging, **local diagnosis was A-PXA. ACNU: nimustine, ChT: chemotherapy. F: female, fr: fraction, F/U: follow-up, IFN: interferon-beta, KPS: Karnofsky performance status, Lt: left, M: male, mo: months, NF1: neurofibromatosis type 1, PCV: procarbazine, lomustine, and vincristine regimen, PR: partial resection, Pre-op: preoperative, PXAs: pleomorphic xanthoastrocytomas, Rec: recurrence, Rt: right, RT: radiotherapy, SRT: stereotactic radiotherapy, STR: subtotal resection, TMZ: temozolomide, TR: total resection.

sequenced on a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with the Big Dye Terminator v.1.1 Cycle Sequencing Kit (Applied Biosystems). Standard procedures were followed.

Statistical analysis

In univariate analyses, log-rank tests were performed to assess the prognostic significance between each parameter and clinical outcomes (PFS and OS). For quantitative parameters, the cutoff points showing the lowest p value were used. Survival curves were generated using the Kaplan–Meier method. Then, the multivariate Cox proportional hazard regression was performed to assess the independence among prognostic factors identified in univariate analyses. P values less than 0.05 were considered significant. All statistical analyses were performed with an EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), the graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, the EZR is a modified version of R commander designed to add statistical functions frequently used in biostatistics.¹⁴⁾

Results

Patient characteristics

The central re-review found four cases who were later diagnosed with tumors other than PXA (ganglioglioma, anaplastic glioma, GBM, and an insufficient sample for diagnosis, respectively) and these patients were subsequently excluded. The remaining 19 cases were investigated in further analysis.

The patient characteristics, radiological findings, and treatment modalities are listed in Table 1 and summarized in Table 2. Of the 19 cases, 11% were anaplastic PXA (A-PXA). There were no gender differences in the results. The median age was 20 years.

Almost all cases had epilepsy or focal signs as initial symptoms. One of the cases was associated with neurofibromatosis type 1, and the lesion in the brain was observed during a general examination. All cases were independent with median preoperative KPS of 90%.

Tumor location was supratentorial in all cases except for one case, and was predominant in the temporal lobe. The median tumor size was as large as 4 cm. Most tumors had mild to moderate peritumoral edema.

Total resection was performed in 11 PXA cases (58%). In this regard, one case received radiochemotherapy according to its local diagnosis: A-PXA. In six PXA cases with subtotal or partial removal, three cases received radiotherapy and one case received chemotherapy. Two A-PXA cases received subtotal removal and postoperative radiochemotherapy.

Survival data were available for all cases, and the median follow-up period was 79 months (range: 9–321 months). In 17 patients with PXA, the 5-year and 10-year PFS rates were 63.5% and 50.8%, respectively. The 5-year and 10-year OS rates were both 88.2%. In two patients with A-PXA, the 5-year PFS and OS rate were both 50%. The 10-year PFS and OS were not determinable. In addition, the median PFS and OS were not determinable.

Histology, immunohistochemistry, and gene mutation findings

The histological and molecular data are listed in Table 3 and summarized in Table 4. Histological features of PXA were characterized by prominent pleomorphism with comparatively less mitosis, vascular proliferation, and necrosis. A-PXA tended to present more aggressive histological features.

Considering the immunophenotypes, all 19 cases were positive or focally positive for GFAP, S-100, and nestin. The stainability of nestin was the most stable.

In neuronal markers, synaptophysin and NFP were positive in 11 (58%) and 9 (47%) of the cases, respectively. Of these, eight cases showed statistically significant rates of co-expression of synaptophysin and NFP ($p=0.0498$, Fisher's exact test).

All the samples were negative for NeuN, a marker of mature neurons, and IDH1 R132H. CD34 was at least focally positive in nine cases (47%). The Ki67 index was generally low, except for A-PXA. The *BRAF* V600E or 599insT mutations were observed in 8/14 cases (57%). The *BRAF* status of 2 A-PXA was wild-type. The *TERT* promoter (*TERTp*) mutation was found in only one case of A-PXA.

Survival analyses

Univariate analyses for PFS revealed that age ≥ 60 years (Fig. 1A), preoperative KPS $\leq 80\%$ (Fig. 1B), and marked peritumoral edema (Fig. 1C) were significant factors associated with tumor recurrence. There was no statistical difference between total resection and subtotal/partial resection (Fig. 1D), and other factors such as tumor size, IHC and mutation status, radiotherapy, and chemotherapy had no prognostic impact for PFS. Multivariate analysis showed no statistically independent prognostic factor: the p value of each factor was 0.073 for age, 0.285 for preoperative KPS, and 0.328 for peritumoral edema, respectively.

Survival analysis for OS was not applicable because only three cases died during the follow-up period.

Table 2 Summary of clinical and radiological characteristics, treatment modalities, and outcomes in the present series

Clinical characteristics	PXA	A-PXA	All
Total number of cases	17	2	19
Male, n (%)	8 (47%)	1 (50%)	9 (47%)
Age at diagnosis, median (range)	20 (7–68)	45 (16–74)	20 (7–74)
Initial symptoms, n (%)			
Seizure	9 (53%)	1 (50%)	10 (53%)
Focal signs	7 (41%)	1 (50%)	8 (42%)
Other	1 (6%)	0	1 (5%)
Preoperative KPS, median (range)	90% (10–100)	80% (70–90)	90% (10–100)
Location, n (%)			
Supratentorial	16 (94%)	2 (100%)	18 (95%)
Temporal lobe	9 (53%)	0	9 (47%)
Infratentorial	1 (6%)	0	1 (5%)
Tumor size*, median (range)	4.0 cm (2.0–8.0)	5.3 cm (3.5–7.0)	4.0 cm (2.0–8.0)
Peritumoral edema, n (%)			
Mild to moderate	13 (76%)	2 (100%)	15 (79%)
Marked	4 (24%)	0	4 (21%)
Extent of resection, n (%)			
Total resection	11 (65%)	0	11 (58%)
Subtotal resection	5 (29%)	2 (100%)	7 (37%)
Partial resection	1 (6%)	0	1 (5%)
Radiotherapy, n (%)	4 (24%)	2 (100%)	6 (32%)
Local radiotherapy	3 (18%)	1 (50%)	4 (21%)
Stereotactic radiotherapy	1 (6%)	0	1 (5%)
Combined	0	1 (50%)	1 (5%)
Chemotherapy, n (%)	2 (12%)	2 (100%)	4 (21%)
Combined with radiotherapy	1 (6%)	2 (100%)	3 (16%)
PFS rate, 5-year/10-year	63.5%/50.8%	50%/not reached	62.2%/49.8%
OS rate, 5-year/10-year	88.2%/88.2%	50%/not reached	84.2%/84.2%

*The longest diameter determined on gadolinium-enhanced T1-weighted magnetic resonance imaging. KPS: Karnofsky Performance Status, PFS: progression-free survival, OS: overall survival.

Discussion

Three outcomes of the present study include, nestin, a neural stem cell marker, exhibits the most robust staining for the diagnosis of PXAs. Second, PXAs demonstrated a low rate of *TERT*p mutation (7%). Finally, prognostic factors for PFS, which are not currently well known, were analyzed statistically.

Regarding nestin, a positive correlation between the level of nestin expression and tumor malignancy has been reported in astrocytic gliomas.^{15,16)} However, little is known in PXAs and the present results may be the first observation to disclose the value of

nestin staining for the diagnosis of PXAs. This may also imply inherent pathological characteristics of PXAs, currently, a theory exists that PXAs possibly arise from multipotent neuroectodermal precursor cells.¹⁾ Nestin is also observed in the mature vascular endothelium.¹⁶⁾ In concert with this, PXAs are often positive for vascular endothelial markers,¹⁷⁾ such as CD34 (47%) in the present series.

The low positive rate of *TERT*p mutation in PXAs was consistent with previously published results.¹⁸⁾ This genetic status was presumed as a molecular feature of PXAs, and may significantly contribute to the practical differential diagnosis between PXAs

Table 3 Histological and molecular data of patients with PXAs

Case	Central review	Pleomor- phism	Mitotic activity	Vascular proliferation	Necrosis	GFAP	S-100	Nestin	Synapto	NFP	NeuN	CD34	IDH1 R132H	Ki67 index (%)	BRAF status*	TERTp status*
1	PXA	++	-	-	-	p	p	p	n	n	n	n	n	0.2		
2	PXA	++	-	-	-	fp	p	p	fp	fp	n	n	n	0.9	wt	wt
3	PXA	++	-	-	-	p	p	p	p	fp	n	fp	n	1.8	V600E	wt
4	PXA	+	-	-	-	p	p	p	p	fp	n	n	n	3.5	599insT	wt
5	PXA	++	-	+	-	p	p	p	n	n	n	n	n	3.7		
6	PXA	++	-	-	-	fp	p	p	n	n	n	fp	n	4.4		
7	PXA	++	-	-	-	p	p	p	fp	p	n	fp	n	0.8		
8	PXA	++	-	-	-	fp	p	p	fp	fp	n	p	n	1.9	V600E	wt
9	PXA	++	-	-	-	p	p	p	fp	n	n	n	n	1.2	wt	wt
10	PXA	+	-	-	-	p	p	p	p	n	n	fp	n	5.2	V600E	wt
11	PXA	++	-	-	-	p	p	p	n	n	n	fp	n	0.9	wt	wt
12	PXA	++	-	-	-	p	p	p	p	n	n	fp	n	2.6		wt
13	PXA	+	+	-	-	p	p	p	fp	fp	n	n	n	2.9	V600E	wt
14	PXA	++	-	-	-	p	p	p	n	fp	n	n	n	1.7	599insT	wt
15	PXA	++	-	-	-	p	p	p	fp	p	n	n	n	4.3	wt	wt
16	PXA	++	-	-	-	p	p	p	NA	n	n	p	n	4	V600E	wt
17	PXA	-	-	-	-	p	p	p	n	NA	n	fp	n	0.3	V600E	wt
18	A-PXA	++	+	+	++	p	fp	p	fp	fp	n	n	n	47.5	wt	wt
19	A-PXA	++	+	+	+	p	p	p	n	n	n	n	n	16.7	wt	C228T

*The results were not obtained for BRAF from five samples and for TERTp from four samples because of degradation of the genome structure. -: none, +: moderate, ++: marked, fp: focally positive, n: negative, NA: not available, p: positive, PXAs: pleomorphic xanthoastrocytoma, synapto: synaptophysin, wt: wild-type.

Table 4 Summary of histological and molecular data in the present series

Light microscopic findings	PXA (17)	A-PXA (2)	All (19)	Immunophenotype	PXA	A-PXA	All
Pleomorphism, n (%)				Synaptophysin, n (%) (data were insufficient in 1 case)			
Marked	13 (76%)	2 (100%)	15 (79%)	Positive	4 (25%)	0	4 (22%)
Moderate	3 (18%)	0	3 (16%)	Focally positive	6 (38%)	1 (50%)	7 (39%)
None	1 (6%)	0	1 (5%)	Negative	6 (38%)	1 (50%)	7 (39%)
Mitotic activity, n (%)				NFP, n (%) (data were insufficient in 1 case)			
Marked	0	0	0	Positive	2 (13%)	0	2 (11%)
Moderate	1 (6%)	2 (100%)	3 (16%)	Focally positive	6 (38%)	1 (50%)	7 (39%)
None	16 (94%)	0	16 (84%)	Negative	8 (50%)	1 (50%)	9 (50%)
Vascular proliferation, n (%)				NeuN, n (%)			
Marked	0	0	0	Negative	17 (100%)	2 (100%)	19 (100%)
Moderate	1 (6%)	2 (100%)	3 (16%)	CD34, n (%)			
None	16 (94%)	0	16 (84%)	Positive	2 (12%)	0	2 (11%)
Necrosis, n (%)				Focally positive	7 (41%)	0	7 (37%)
Marked	0	1 (50%)	1 (5%)	Negative	8 (47%)	2 (100%)	10 (53%)
Moderate	0	1 (50%)	1 (5%)	IDH1, n (%)			
None	17 (100%)	0	17 (90%)	Negative	17 (100%)	2 (100%)	19 (100%)
Immunophenotype				Ki67 index			
GFAP, n (%)				Median (range)	1.9% (0.2–5.2)	32.1% (16.7–47.5)	2.6% (0.2–47.5)
Positive	14 (82%)	2 (100%)	16 (84%)	Mutation status			
Focally positive	3 (18%)	0	3 (16%)	<i>BRAF</i> , n (%) (data were insufficient in 5 cases)			
Negative	0	0	0	V600E	6 (50%)	0	6 (43%)
S-100, n (%)				599insT	2 (17%)	0	2 (14%)
Positive	17 (100%)	1 (50%)	18 (95%)	Wild-type	4 (33%)	2 (100%)	6 (43%)
Focally positive	0	1 (50%)	1 (5%)	<i>TERT</i> promoter, n (%) (data were insufficient in 4 cases)			
Negative	0	0	0	C228T	0	1 (50%)	1 (7%)
Nestin, n (%)				Wild-type	13 (100%)	1 (50%)	14 (93%)
Positive	17 (100%)	2 (100%)	19 (100%)				
Focally positive	0	0	0				
Negative	0	0	0				

PXA: pleomorphic xanthoastrocytoma.

and GBMs including giant cell glioblastoma (gcGBM) and epithelioid glioblastoma (eGBM). GBMs, IDH-wild-type, and eGBMs show a high frequency of *TERT*p mutations (71–90%).^{3,10,19–21} This enables differentiation from PXAs. Since GBMs, IDH-mutant and gcGBMs, have a low rate of *TERT*p mutation (28% and 25%, respectively).^{22,23} The differentiation from PXA is therefore not feasible by only *TERT*p mutation. However, the present and previous studies

indicate that *IDH* mutation is absent in PXAs.^{4,18,24} In addition, neuronal markers that are often positive in PXAs are negative in gcGBM.²⁵ Further, whereas 50%–78% of PXAs present in *BRAF* V600E mutation,¹ the frequency is less than 10% in IDH-mutant GBM and gcGBM.¹³ Thus, there is a possibility that the mutation status of *TERT*p is a significant distinctive element between PXAs and GBMs, as also supported by the results of the present study.

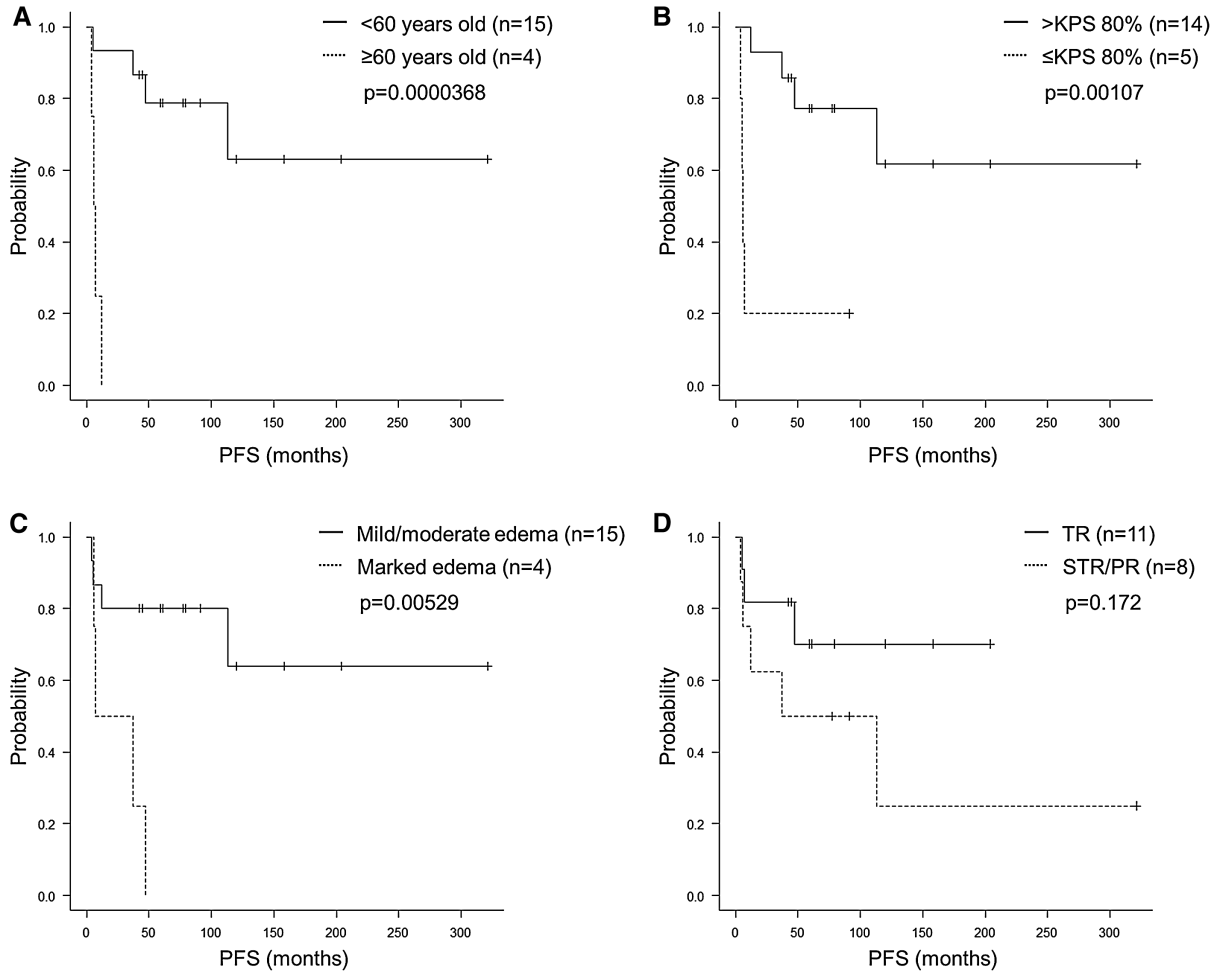


Fig. 1 Representative Kaplan-Meier survival curves for PFS according to age (<60 years vs. ≥60 years) (A), preoperative KPS (>80% vs. ≤80%) (B), peritumoral edema on MRI (mild to moderate vs. marked) (C), and extent of resection (TR vs. STR and PR) (D). KPS: Karnofsky performance status, PFS: progression-free survival, PR: partial resection, STR: subtotal resection, TR: total resection.

The last point concerns the prognostic factors of PXAs. The present survival analyses demonstrated that advanced age, poor preoperative KPS, and marked perifocal edema were significantly associated with a shorter PFS. These results are consistent with previous studies suggesting that higher age²⁶⁾ and marked perifocal edema²⁷⁾ are associated with worse PFS in PXAs. The KPS has not been well investigated in available literature and the present results may provide a new insight.

Limitations of the present study include a small number of patients and retrospective nature that leads to inherent biases. Nevertheless, this study may help clinicians make proper diagnoses and prognostic prediction, and encourage a larger clinical study.

The present study analyzed 19 patients with PXA retrospectively gathered from seven universities of the Tohoku district of Japan over a 21-year period. In molecular features, PXA was characterized by positive nestin and few *TERT* mutation. Neuronal markers may help diagnosis. Several prognostic factors were identified on univariate analysis, but patient age and preoperative KPS were the strongest factors. A further clinical study may be required.

Acknowledgments

The authors would like to sincerely thank Dr. Masaya Oda, Dr. Ryuta Saito, Dr. Yuichi Sato, and Dr. Kaori Sakurada for their constructive suggestions for this study.

The research was supported by Research Grant of Japan Brain Foundation, 2014.

Conflicts of Interest Disclosure

All authors report no conflicts of interest concerning this article.

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