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Molecular Subgrouping Based On Immunohistochemistry In Medulloblastoma: A Single-Center Experience

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ABSTRACT

AIM: To investigate the efficacy of immunohistochemical methods to determine molecular subgroups and prognostic predictions of medulloblastomas (MBs).

MATERIAL and METHODS: β-catenin, GAB1, YAP1, filamin A and p53 were immunohistochemically stained, and MYC and MYCN fluorescent in situ hybridization (FISH) procedures were applied to 218 cases in our series.

RESULTS: Based on the histomorphological characteristics of the cases, 67.9% were deemed classic MB; 15.6% as desmoplastic/nodular medulloblastoma (DNMB); 12.8% as large cell/anaplastic (LC/A) MB; 3.7% as medulloblastoma with extensive nodularity (MBEN). Molecular characteristics revealed that 50.5% had non-WNT/non-SHH; 33.9% had SHH-activated and TP53-wildtype; 8.7% had WNT-activated; 6.9% had SHH-activated and TP53-mutant. According to the survival curves, LC/A MBs or non-WNT/non-SHH tumors showed the worst prognosis, whereas DNMBs and WNT-activated tumors showed the best prognosis. Classic MBs or SHH-activated tumors showed a moderate course. MYCN amplification was found to act as an independent poor prognostic factor in the study.

CONCLUSION: The distribution of histological subtypes and molecular subgroups, amplification rates, and prognostic data obtained through immunohistochemical methods in our study were consistent with those reported in the literature. It was therefore hypothesized that the determination of molecular subgroups by immunohistochemical methods can be useful in daily diagnostic practice, especially in centers with limited access to molecular techniques.

KEYWORDS: Histopathology, Immunohistochemistry, Medulloblastoma, Molecular Subgrouping, Prognosis

ABBREVIATIONS: MB: Medulloblastoma, **DNMB:** Desmoplastic/nodular medulloblastoma, **MBEN:** Medulloblastoma with extensive nodularity, **LC/A MB:** Large cell/anaplastic medulloblastoma, **FISH:** Fluorescent in situ hybridization, **NGS:** Next-generation sequence, **SHH:** Sonic-Hedgehog, **WHO:** World Health Organization

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INTRODUCTION

Medulloblastomas (MBs), usually observed in childhood, are the most common primary malignancy of the central system in the pediatric age group and also the most common brain tumor after pilocytic astrocytoma (2,9,12,19). Similar to other embryonal central nervous system tumors, it can classified as grade 4 according to the World Health Organization (WHO) guidelines (1,3,10,15,16). In line with the recent developments in molecular pathology, the 2016 and 2021 WHO classifications proposed the use of molecular subgroups based on gene expression and methylation profiles. Accordingly, the four genetic subgroups were identified as WNT-activated, SHH-activated and TP53-wild type, SHH-activated and TP53-mutant and non-WNT/non-SHH. Non-WNT/non-SHH medulloblastomas comprise group 3 and group 4 tumors (3,16,17). In a meta-analysis based on methylation profiles, SHH and non-WNT/non-SHH medulloblastomas could be further classified into four and eight molecular subgroups respectively (3,27).

However, these new molecular subgroups based on transcriptional methylation profiles and gene expression are not easily applicable or affordable in practice and can further deepen disparities between high and low/middle income countries (29). Therefore, pathologists have sought alternatives that can be readily applied, particularly in the field of immunohistochemistry (5,21).

This study aimed to elucidate the efficacy of immunohistochemical methods to detect molecular subgroups as well as to correlate the determined genetic subgroups with histological subtypes and prognosis. We have reported here our experience at our center.

MATERIAL and METHODS

Study Tumor Cohort

The study tumor cohort comprised 218 cases diagnosed with medulloblastoma in our department from 1981 to 2015 (This study was conducted with appropriate ethics committee approval: Hacettepe University Non-interventional Research Ethics Committee; 16/160). The evaluation was performed by two pathologists, one of whom is an experienced neuropathologist (FS). Age, sex, biopsy and file number, date of surgery, recurrence status, date of death, and histopathological tumor types listed in the report were recorded. In addition, data on whether the surgery was total or subtotal was obtained from the clinical files, and data on metastatic disease at the time of presentation and risk classification were also collected. The cases were classified histopathologically according to the WHO guidelines as classic MB, desmoplastic/nodular medulloblastoma (DNMB), medulloblastoma with extensive nodularity (MBEN), and large cell/anaplastic (LC/A) MB (Figure 1). Based on the pediatric oncological risk classification, patients younger than 3 years, patients with metastatic disease at presentation, and patients who underwent subtotal surgery were placed in the high-risk category, while the remaining were classified as the average risk category.

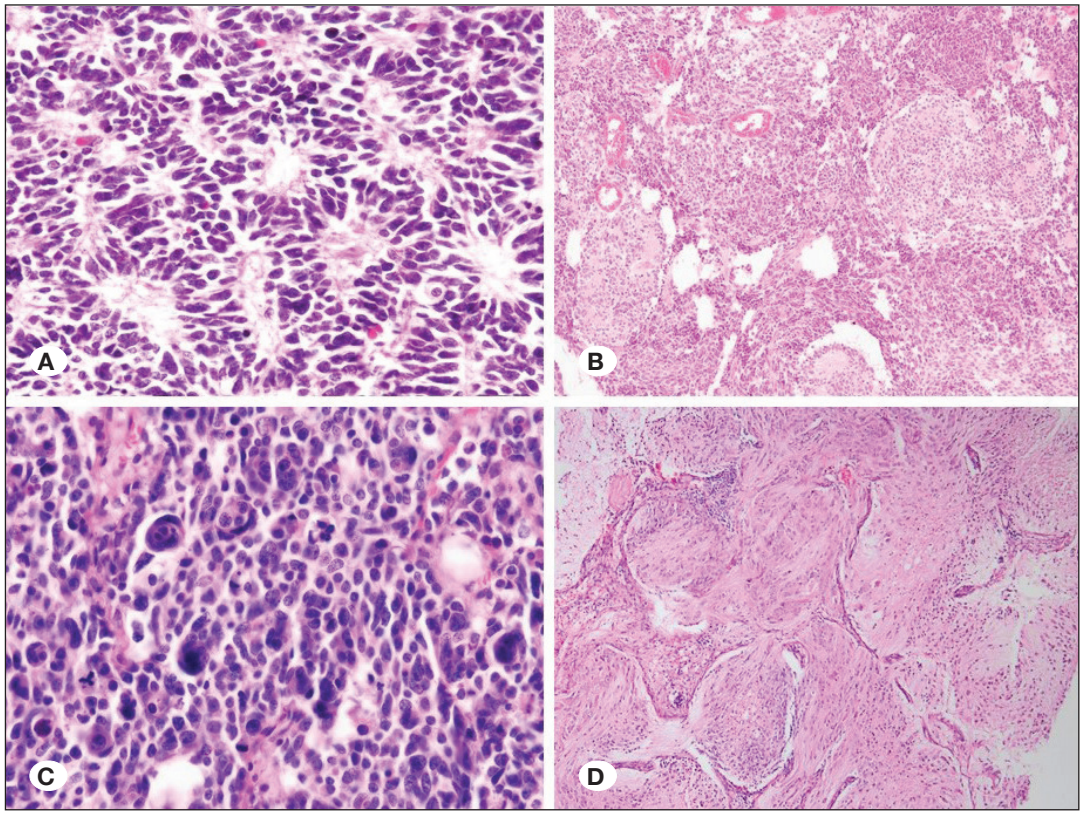

Figure 1: Histopathological characteristics of medulloblastomas (MBs); classic MB with multiple Homer-Wright rosettes and rhythmic, palisade-like appearance **(A)** desmoplastic/nodular MB (DNMB) characterized by well-differentiated nodules and surrounded by more cellular areas **(B)** large cell/anaplastic MB (LC/A MB), characterized by marked pleomorphism, “molding” and “cell wrapping” **(C)** MB with extensive nodularity (MBEN), characterized by large nodules and poorly differentiated tumor cells in the surrounding desmoplastic matrix **(D)**.

Immunohistochemical Examination and Evaluation

Tissue microarray blocks (TMA) with a core size of 4 mm were stained with hematoxylin and eosin, and immunohistochemical staining was performed on the automated Leica BOND-MAX IHC/ ISH immunostainer using the following commercial antibodies: β-catenin (1:400, Biocare), GAB1 (1:400, Gene Tex - GTX111253), YAP1 (1:400, Gene Tex - GTX129151), filamin A (1:400, Gene Tex - GTX61826), and p53 (1:200, Biocare).

In this study, as stated in the literature, MBs with more than %5 of cells showing nuclear positivity for β-catenin along with YAP1 immunopositivity were considered WNT-activated. If <5% of the neoplastic cells had nuclear or cytoplasmic positivity or no staining for β-catenin, the tumors were accepted as falling outside the WNT category (8,11). MBs with combined immunoreactivities for GAB1, YAP1 and filamin A, were accepted as SHH-activated (5). MBs displaying immunonegativity for GAB1 and YAP1 as well as cytoplasmic immunoreactivity for β-catenin were categorized as the non-WNT/non-SHH molecular subgroup. Nuclear expression in more than 50% of the cells for p53 or the complete loss of expression were considered TP53 mutant, and staining between 1% and 50% was considered in the wild type (13) (Figure 2). GAB1, YAP1 and filamin A were considered significant when they stained positively in more than half of the neoplastic cells.

Fluorescent in Situ Hybridization (FISH)

ZytoLight SPEC MYCN/2q11 Dual Color Probe and ZytoLight SPEC MYC Dual Color Break Apart Probe were employed in the FISH studies on N-myc and C-myc. Two red and two green signals per cell were considered normal; if the cells with ≥10 signals or numerous tight signal clusters accounted for ≥10% of the tumor cells, they were considered to indicate amplifications (Figure 3).

Statistical Analysis

The data of 218 cases were transferred to the IBM-SSPS 23 medical statistics program. Descriptive statistics were presented as the mean ± SD for parametric continuous variables, median for nonparametric continuous variables and

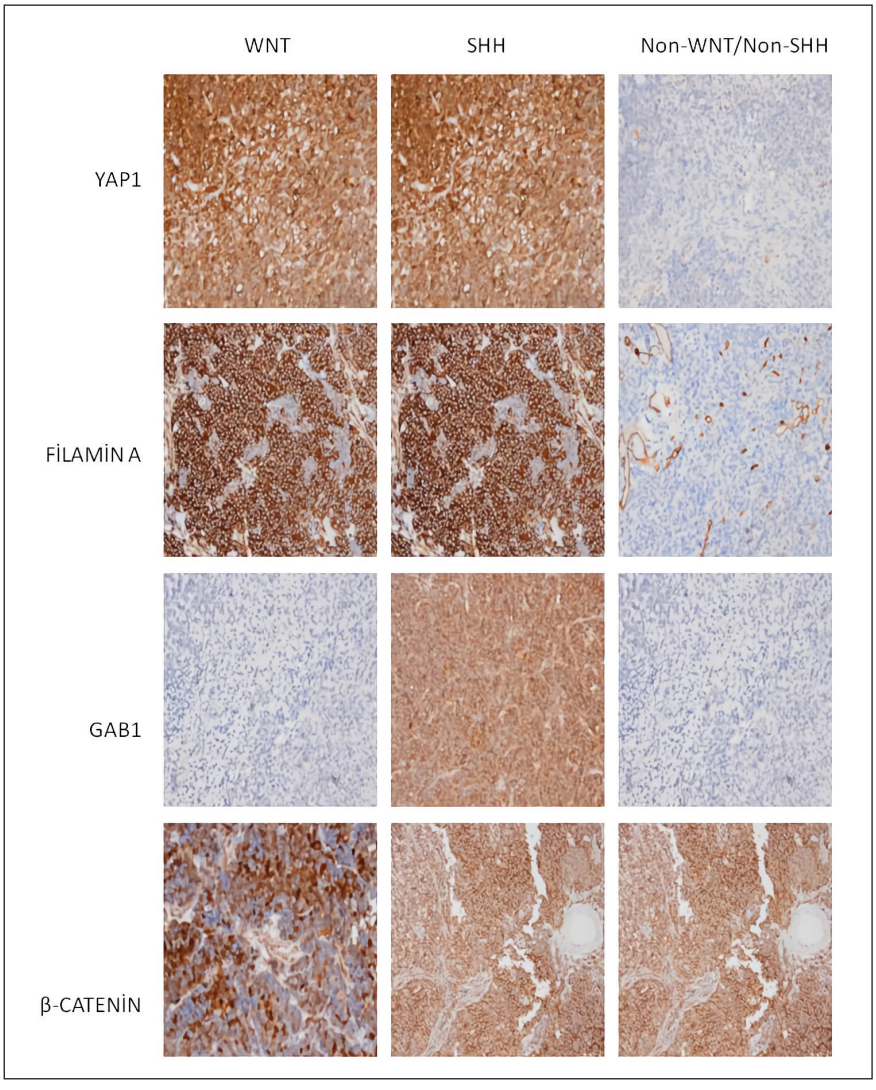

Figure 2: Determination of molecular subgroups by immunohistochemical methods.

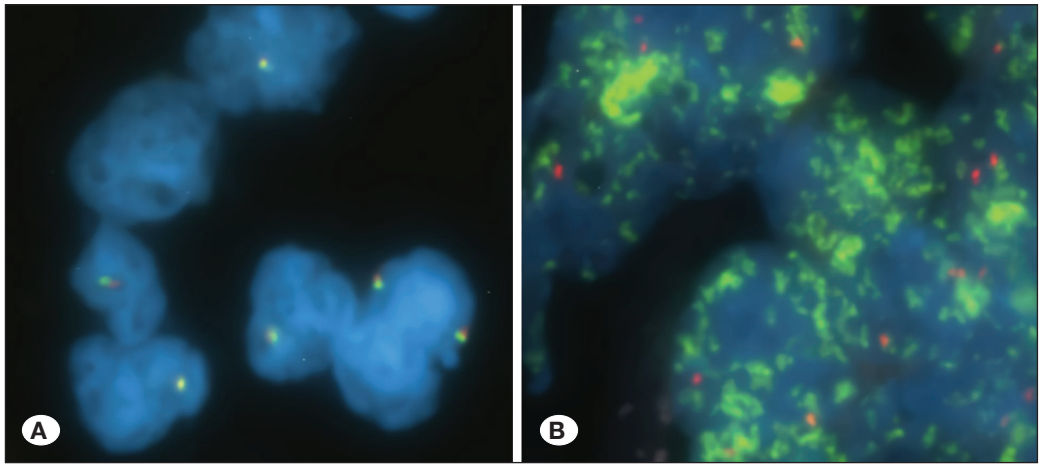

Figure 3: Normal **(A)** and amplified **(B)** samples for N-myc and C-myc; two red and two green signals per cell are considered normal; cells with ≥10 signals or numerous tight signal clusters were considered amplification if they accounted for ≥10% of the tumor cells.

Table I: Distribution of Molecular Subgroups Based on Immunohistochemistry by Histological Subtypes

	WNT-activated	SHH-activated and TP53-mutant	SHH-activated and TP53-wildtype	Non-WNT/ Non-SHH	n (%)
Classic MB	13	6	27	102	148 (67.8)
DNMB	0	4	30	0	34 (15.6)
MBEN	0	1	7	0	8 (3.7)
LC/A MB	6	4	10	8	28 (12.8)
n (%)	19 (8.7)	15 (69)	74 (33.9)	110 (50.5)	218

MB: *Medulloblastoma,* ***DNMB:*** *Desmoplastic/nodular medulloblastoma, MBEN: Medulloblastoma with extensive nodularity,* ***LC/A MB:*** *Large cell/anaplastic medulloblastoma,* ***SHH:*** *Sonic-Hedgehog.*

percentage for categoric variables. Fisher's exact test and (Fisher-Freeman-Halton) test were employed to examine the association between qualitative variables. The survival curves were generated using Kaplan-Meier analysis to calculate the survival times. Correlations between the survival times and several other variables were examined univariately with the log-rank test. For multivariate survival analysis, the backward variable elimination technique and Cox proportional hazard regression analysis were applied. For all statistical tests, a fallibility (α) of 0.05 was assumed, and $p \leq 0.05$ was considered to indicate statistical significance.

RESULTS

General Results

According to the pediatric oncological risk classification, 144 (66.1%) cases belonged to the standard risk and 74 (33.9%) to the high-risk categories. Of these cases, 133 (61%) were male and 85 (39%) were female. The mean age of the patients at the time of initial diagnosis was 11.6 ± 9.2 years (age range: 6 months and 51 years). Patients were followed up for a minimum of 43 months and a maximum of 206 months, excluding those who died. The median follow-up time of patients was 69 months.

Histomorphological and Molecular Findings

The histological subtypes in our series were predominantly classic (67.9%) MB, followed by a decreasing order of DNMB (15.6%), LC/A MB (12.8%), and MBEN (3.7%). Molecular subgroups were determined as non-WNT/non-SHH (50.5%), SHH-activated and TP53-wildtype (33.9%), WNT-activated (8.7%) and SHH-activated, and TP53-mutant (6.9%).

When the molecular subgroup distribution of histological subtypes was examined, it was noted that all DNMB and MBEN cases were evaluated as SHH-activated tumors. LC/A MBs were observed most frequently (35.7%) in the SHH-activated and TP53-mutant tumors. In addition, most of the classic MBs (68.9%) were deemed non-WNT/non-SHH tumors (Table I).

According to the histological distribution of molecular subgroups, WNT-activated tumors included classic or LC/A MBs; SHH-activated tumors included all four histological spectra of MBs whereas non-WNT/non-SHH tumors were either classic or LC/A MBs. In addition, 68.4% of the WNT-activated tumors displayed a classic morphology and 31.6% displayed a LC/A morphology. All DNMB and MBEN were SHH-activated tumors and were frequently TP53-wildtype (88% and 87.5%, respectively). Among non-WNT/non-SHH tumors, classic morphology was observed in 92.7% and LC/A morphology in 7.3% of the cases.

Twenty (9.2%) out of 218 patients were TP53-mutant and 198 (90.8%) patients were TP53-wild type. TP53-mutant tumors were observed in 4 (14.3%) of LC/A MB, 1 (12.5%) of MBEN, 4 (11.8%) of DNMB, and 11 (7.4%) of classic MB cases. Although TP53 mutation was relatively common among LC/A MB, there was no statistically significant difference in the distribution of histological subtypes (p=0.636). The examination of the molecular subgroups distribution of 20 patients with TP53-mutant revealed that 15 (75%) patients were SHH-activated, 3 (15%) patients were WNT-activated and 2 (10%) patients were non-WNT/non-SHH.

MYCN amplification was detected in 11 (5%) patients and MYC amplification in 19 (8.7%) patients. MYCN and MYC amplifications were mutually exclusive. MYC amplified tumors were observed in 11 (57.9%) of classic MB, 7 (36.8%) of LC/A MB, and 1 (5.3%) of DNMB but not in MBEN (p=0.008). Like MYC amplification, MYCN amplification was observed in 5 (45.5%) of classic MB, 4 (36.4%) of LC/A MB, and 2 (18.2%) of DNMB cases, but not in MBEN (p=0.81). MYCN amplification was observed in 6 (54.5%) of SHH-activated and TP53-wild type, 4 (36.4%) of non-WNT/non-SHH and 1 (9.1%) of SHH-activated and TP53-mutant tumors, while MYCN amplification was not detected in WNT-activated tumors (p=0.441). MYC amplification was significantly detected in the non-WNT/non-SHH group (n=10 patients, 52.6%), but was observed to varying degrees in all molecular subgroups. (p=0.006). Five of 19 (26.3%) patients with MYC amplification were identified to have WNT-activated tumors.

Survival Results

Classification of patients by the age of 3 years, defined as the threshold age in the pediatric oncology risk classification, indicated that the median survival time was 12 months in patients younger than 3 years and 99 months in patients older than 105 years of age (p<0.001).

Because more than half of the female patients were still alive, the median survival time could not be calculated. Among the male patients, this duration was 52 months. The impact of female gender on the overall survival rate was found to be statistically significant (p=0.014).

The median survival time of patients with metastatic disease at the time of presentation was 63 months. Because more than half of the patients without metastases were still alive, the median survival time could not be calculated. The median life expectancy of these patients was 138 months. Metastatic disease at presentation was considered a significant poor prognostic factor in the analysis of overall survival (p=0.011). Although the number of patients with clinical follow-up was limited, a lower rate of metastatic disease at the time of presentation was observed in DNMB relative to that in classic and LC/A MBs (p=0.045). Furthermore, none of the WNT-activated tumors had metastatic disease at the time of presentation, while non-WNT/non-SHH tumors had the highest number of metastatic disease (p=0.042).

In our series, the median survival time among histological subtypes was 105 months for MBEN, 33 months for LC/A MB, and 56 months for classic MB. Because more than half of the DNMB patients were still alive, the median survival time could not be calculated (p=0.450) (Figure 4).

When assessing the impact of molecular subgroups on prognosis, the median survival time was 115 months for SHH-activated and TP53-wildtype tumors, 52 months for non-WNT/non-SHH tumors, and 33 months for SHH-activated and TP53-mutant tumors. Since more than half of the patients with the WNT-activated tumors were still alive, the median survival rate could not be calculated (p=0.155) (Figure 4).

Although TP53 mutation was not determined to be a significant prognostic factor in our series (p=0.553), it is noteworthy that the SHH-activated and TP53-mutant tumors showed a higher rate of mortality and recurrence than the SHH-activated and TP53-wildtype tumors.

The overall survival time was 19 months for the cases with MYCN amplification, while it was 63 months for the non-amplified cases. MYCN amplification was a poor prognostic factor in univariate analysis of overall survival (p=0.026). The median survival time was 24 months in cases with MYC amplification and 73 months in the non-amplified cases. MYC amplification was not a poor prognostic factor in univariate overall survival analysis (p=0.059).

Tumors in the average risk category were significantly associated with lower recurrence and mortality rates compared to tumors in the high-risk category. Thirty-four (23.6%) of the 144 patients in the average risk category and 39 (52.7%) of the 74 patients in the high-risk category died (p<0.05). In 29 (20.1%) of the 144 patients in the average risk category and 29 (39.2%) of the 74 patients in the high-risk category, recurrence was observed during the follow-up (p<0.05).

The progression-free survival rates of all cases for 2, 5 and 10 years, respectively were 63%, 52%, and 38% (Table II).

Univariate analysis revealed that age, gender, MYCN amplification, and metastatic disease at the time of presentation were statistically significant factors for the overall survival time. Accordingly, age ≤3 years, male gender, MYCN amplification, and metastatic disease at presentation were poor prognostic factors (Table III).

Multivariate Cox proportional hazard regression analysis performed by the backward variable elimination method indicated that the factors that most strongly affected the overall survival were age, sex and histological subtype. Age ≤3 years (p=0.088; hazard ratio=2.911) and male gender (p=0.077; hazard ratio=1.812) were poor prognostic factors, whereas MBEN histological subtype was a good prognostic factor (p=0.089; hazard ratio=0.261).

DISCUSSION

Developments in the field of molecular studies, the discovery of new mutations, and the determination of gene expression and methylation profiles have ensured the elucidation of genetic causes in neuropathology, particularly MB to develop current treatment modalities and to gain access to reliable prognostic data. For this purpose, numerous studies have

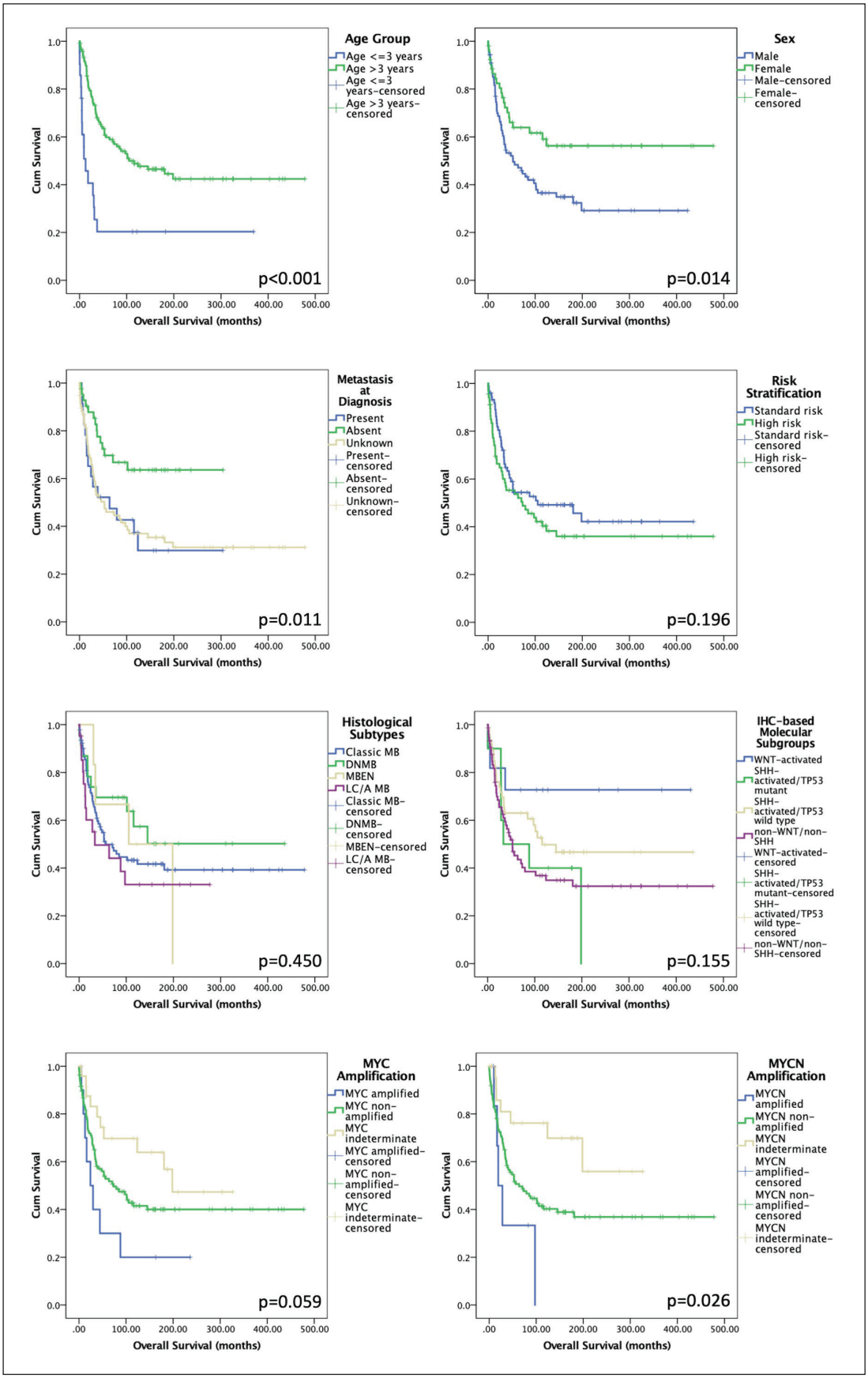

Figure 4: Kaplan-Meier curves for overall survival according to age, sex, metastasis at diagnosis, risk stratification groups, histopathological subtypes, molecular subgroups and MYCN ve MYC amplification.

Table II: Progression Free-Survival Rates in Medulloblastoma for Histological Subtypes and Molecular Subgroups

	2 years (%)	5 years (%)	10 years (%)
Classic MB	63	49	38
DNMB	70	70	46
MBEN	67	67	25
LC/A MB	50	44	33
WNT-activated	82	73	73
SHH-activated and TP53-mutant	50	50	24
SHH-activated and TP53-wild type	63	63	44
Non-WNT/Non-SHH	61	42	30
All cases	63	52	38

MB: *Medulloblastoma,* ***DNMB:*** *Desmoplastic/nodular medulloblastoma, MBEN: Medulloblastoma with extensive nodularity,* ***LC/A MB:*** *Large cell/anaplastic medulloblastoma,* ***SHH:*** *Sonic-Hedgehog.*

Table III: Variables Found to be Significant for Survival in Univariate Analyses

	Subgroups	OS (median) (month)	p-value
Age at diagnosis	≤3	12	**<0.001**
	>3	105	
Sex	Female	NA	**0.014**
	Male	52	
Histopathological subtypes	Classic MB	56	0.450
	DNMB	NA	
	MBEN	105	
	LC/A MB	33	
Molecular subgroups	WNT-activated	NA	0.155
	SHH-activated and TP53-mutant	33	
	SHH-activated and TP53-wildtype	115	
	Non-WNT/Non-SHH	52	
TP53 mutation	Wildtype	35	0.553
	Mutant	25	
MYCN amplification	Negative	63	**0.026**
	Positive	19	
MYC amplification	Negative	73	0.059
	Positive	24	
Metastatic disease at the time of presentation	M-	NA	**0.011**
	M+	63	
Surgery	Gross total	47	0.123
	Subtotal	143	
Pediatric risk group	Average-risk	105	0.196
	High-risk	73	

log-rank chi-square test.* ***NA:* *Median survival cannot be calculated because ≥50% of cases are still alive.* ***MB:*** *Medulloblastoma,* ***DNMB:*** *Desmoplastic/nodular medulloblastoma, MBEN: Medulloblastoma with extensive nodularity,* ***LC/A MB:*** *Large cell/anaplastic medulloblastoma,* ***SHH:*** *Sonic-Hedgehog.*

been conducted, particularly in the past 15 years, to discover the molecular mechanism and to determine the molecular subgroups (6,11,20).

The use of molecular subgroups has been recommended instead of the approach based only on histomorphological classification. As a result of all these studies, the WHO Classification of Central Nervous System Tumors was published in 2016 and 2021 (3,16). It was emphasized that in addition to the four histological subtypes such as classic MB, DNMB, MBEN, and LC/A MB, four molecular subgroups of MBs such as WNT-activated, SHH-activated and TP53-mutant, SHH activated and TP53-wildtype and non-WNT/non-SHH should be defined. Non-WNT/non-SHH medulloblastomas comprise groups 3 and groups 4 tumors (3,16).

However, in developing countries like ours, molecular methods are not available in all laboratories. For this reason, the determination of these subgroups by immunohistochemical methods, which is a more feasible method, is useful for patient follow-up and potential treatment modalities.

The first of the studies that proposed this alternative molecular approach was a study by Nortcott et al, wherein the authors analyzed 294 MB cases for their gene expression profiles and DNA copy numbers by using an immunohistochemical panel consisting of DKK1, SFRP1, NPR3, and KCNA1 (21). Subsequently, 22 MB subgroup-specific genes were defined by next-generation sequencing (NGS) (22). However, both these studies emphasized that molecular methods face difficulty in getting accepted due to the related financial constraints, especially in developing countries.

Meanwhile, a study by Ellison et al. suggested that WNT-activated, SHH-activated, and non-WNT/non-SHH subgroups can be practically determined with the use of a panel of immunohistochemical markers (such as YAP1, GAB1, filamin A, and β-catenin) (5).

In the most comprehensive series (Ellison) using immunohistochemical methods in the literature, histological classification of MBs was reported as 72% for classic MB, 17% for desmoplastic MB (including DNMB and MBEN), and 10% for LC/A MB. In the molecular classification of MBs in this study, 55% of cases were non-WNT/non-SHH, 31% were SHH-activated, and 14% were WNT-activated (6).

In our study, we defined the molecular subgroups of 218 cases, diagnosed as MB according to their histomorphological characteristics, using immunohistochemical markers, YAP1, GAB1, filamin A, β-catenin, and p53. We investigated MYCN and MYC amplification in all cases by using the FISH method. Finally we analyzed the impact of all findings on the overall survival.

However, the major shortcoming of our study is that we do not have sufficient technical capabilities to confirm the molecular subgroups with methylation profiles, cytogenetic studies, or next-generation sequencing. Nevertheless, both the distribution rates and prognostic data of molecular subgroups obtained by the immunohistochemical panel were found to be consistent with the literature reports.

The histological subtypes of MB are the most crucial factor contributing to prognosis (18). Desmoplastic MBs (DNMB and MBEN) have the best prognosis, followed by classic MB and LC/A MB, respectively (18). According to the impact of molecular subgroups on prognosis, WNT-activated tumors have a good prognosis, SHH-activated and group 4 tumors have a moderate prognosis, and group 3 tumors have a poor prognosis (4-7,11,28). Moreover, by histological subtype, DNMB and MBEN showed a good, classic MB showed a moderate, and LC/A MB showed the worst prognosis in our series. In the prognostic analysis of molecular subgroups, WNT-activated tumors had a good prognosis, SHH-activated and TP53-wildtype tumors had a moderate prognosis, while SHH-activated and TP53-mutant tumors and non-WNT/non-SHH tumors had a poor prognosis.

WNT-activated tumors mostly exhibited classic morphology, and to a lesser extent, LC/A morphology. Conversely, non-WNT/non-SHH tumors displayed classic or LC/A morphology. All DNMB and MBEN are SHH-activated tumors. SHH-activated tumors displayed classic and LC/A morphology, except for desmoplastic MBs. Because of their prognostic aspect, SHH-activated tumors are screened for TP53 mutation, and remarkably, LC/A morphology is dominant in the TP53-mutant group (6,16,20). Consistent with the literature, in our series, we found that all DNMB and MBEN were observed in the SHH-activated subgroup. Most of the classic MBs (68.9%) belonged to the non-WNT/non-SHH tumors category. LC/A MBs were observed to be the most frequent (35.7%) among the SHH-activated and TP53-mutant subgroups.

As per the literature, WNT-activated tumors, group 3 tumors, and to a lesser extent SHH-activated tumors show MYC amplification, while SHH-activated tumors show MYCN amplification. MYCN and MYC amplifications were recorded to be rare in group 4 tumors. Regardless of the histological subtypes and molecular subgroups, MYCN and MYC amplifications have been associated with poor prognosis (4-6,8,11, 20,24,26). In our study, MYCN amplification was observed in non-WNT/non-SHH tumors and SHH-activated tumors, but not in WNT-activated tumors. Alternatively, MYC amplification was observed to varying degrees in all molecular subgroups, and it was significantly common in non-WNT/non-SHH tumors. As in our study, MYC amplification in SHH-activated tumors has also been reported in the literature, although to a lesser extent than in group 3 and WNT-activated tumors (6, 25). MYCN and MYC amplifications are also mutually exclusive, as described in the literature (23).

Although a relatively compatible graph with the literature was drawn, no statistically significant result could be obtained when the overall survival analyses of the molecular subgroups were considered in our study owing to the proportional lack of patients with clinical follow-up. However, contrary to expectations (4-7,11,28), patients with non-WNT/non-SHH tumors showed longer median survival than SHH-activated and TP53-mutant tumors. Detailed examination of the impact of molecular subgroups on prognosis revealed that age is also an important factor in this aspect. While SHH-activated

tumors showed a better prognosis in infants, the literature reports that they can be worse in children and adults (14,26). Therefore, the prognosis of non-WNT/non-SHH tumors varies with the age of the patient.

CONCLUSION

Among the general prognostic data of our study, ≤3 years, male gender, the presence of MYCN amplification, and metastatic disease at the time of presentation stood out as poor prognostic factors, while MBEN emerged as a good prognostic factor.

It is difficult to establish molecular methods in developing countries, therefore it is critical to determine molecular subgroups by immunohistochemical methods. Histopathological subtypes and molecular subgroups and prognostic MYCN, MYC amplification and TP53 mutation status should be reported in pathology reports whenever possible. In addition to the integrated diagnosis, the indication of risk classification based on age, metastatic disease at the time of presentation, and the presence of residual postoperative tumor are valuable in providing concrete prognostic information for clinic application.

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AUTHORSHIP CONTRIBUTION

Study conception and design: BB, FS
Data collection: SH, AV, KKO, BB
Analysis and interpretation of results: BB, SH, AD
Draft manuscript preparation: BB
Critical revision of the article: BB, SH, FS
All authors (BB, SH, AV, KKO, BB, AD, FS) reviewed the results and approved the final version of the manuscript.

REFERENCES

1. Akyuz C, Varan A, Kupeli S, Akalan N, Soylemezoglu F, Zorlu F, Kutluk T, Buyukpamukcu M: Medulloblastoma in children: A 32-year experience from a single institution. J Neurooncol 90:99-103, 2008
2. Bilginer B, Hanalioglu S, Turk CC, Narin F, Oguz KK, Soylemezoglu F, Akalan N: Is the knowledge pertaining to adult glioblastomas enough for pediatric cases? Prognostic factors in childhood. Turk Neurosurg 27:279-288, 2017
3. Board WCoTE: Central Nervous System Tumours. International Agency for Research on Cancer, 2022
4. Cho YJ, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, Berhoukim R, Amani V, Goumnerova L, Eberhart CG, Lau CC, Olson JM, Gilbertson RJ, Gajjar A, Delattre O, Kool M, Ligon K, Meyerson M, Mesirov JP, Pomeroy SL: Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. J Clin Oncol 29:1424-1430, 2011
5. Ellison D: Methods and compositions for typing molecular subgroups of medulloblastoma. Google Patents, Publication of WO 2012/044921 A1, 2015
6. Ellison DW, Dalton J, Kocak M, Nicholson SL, Fraga C, Neale G, Kenney AM, Brat DJ, Perry A, Yong WH, Taylor RE, Bailey S, Clifford SC, Gilbertson RJ: Medulloblastoma: Clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. Acta Neuropathol 121:381-396, 2011
7. Ellison DW, Kocak M, Dalton J, Megahed H, Lusher ME, Ryan SL, Zhao W, Nicholson SL, Taylor RE, Bailey S, Clifford SC: Definition of disease-risk stratification groups in childhood medulloblastoma using combined clinical, pathologic, and molecular variables. J Clin Oncol 29:1400-1407, 2011
8. Goschzik T, Zur Mühlen A, Kristiansen G, Haberler C, Stefanits H, Friedrich C, von Hoff K, Rutkowski S, Pfister SM, Pietsch T: Molecular stratification of medulloblastoma: Comparison of histological and genetic methods to detect Wnt activated tumours. Neuropathol Appl Neurobiol 41:135-144, 2015
9. Hasbay B, Kayaselcuk F, Suner HI, Sarialioglu F: Medulloblastoma: Clinicopathological correlates of SHH, WNT, and Non-WNT/SHH molecular subgroups analysis and prognostic significance: Mono-institutional series. Turk Neurosurg 32:595-602, 2022
10. Isikay I, Hanalioglu S, Basar I, Narin F, Bilginer B: Survival benefit with gross total resection and adjuvant radiotherapy in childhood atypical teratoid/rhabdoid tumors: Results of a single-center cohort of 27 cases. Turk Neurosurg 29:689-697, 2019
11. Kaur K, Kakkar A, Kumar A, Mallick S, Julka PK, Gupta D, Suri A, Suri V, Sharma MC, Sarkar C: Integrating molecular subclassification of medulloblastomas into routine clinical practice: A simplified approach. Brain Pathol 26:334-343, 2016
12. Kaya IS, Aksoy S, Mutlu M, Tekin C, Taskapilioglu MO, Tunca B, Civan MN, Ocak PE, Kocaeli H, Bekar A, Egeli U, Cecener G, Tolunay S: Comparison of clinical and molecular Wnt and SHH subgroups in medulloblastoma tumor cases. Turk Neurosurg 31:554-565, 2021
13. Köbel M, Reuss A, du Bois A, Kommoss S, Kommoss F, Gao D, Kalloger SE, Huntsman DG, Gilks CB: The biological and clinical value of p53 expression in pelvic high-grade serous carcinomas. J Pathol 222:191-198, 2010
14. Kool M, Korshunov A, Remke M, Jones DT, Schlanstein M, Northcott PA, Cho YJ, Koster J, Schouten-van Meeteren A, van Vuurden D, Clifford SC, Pietsch T, von Bueren AO, Rutkowski S, McCabe M, Collins VP, Bäcklund ML, Haberler C, Bourdeaut F, Delattre O, Doz F, Ellison DW, Gilbertson RJ, Pomeroy SL, Taylor MD, Lichter P, Pfister SM: 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 123:473-484, 2012
15. Louis D: WHO Classification of Tumors of the Central Nervous System, 2007
16. Louis DN: WHO Classification of Tumors of the Central Nervous System, 2016

17. Louis DN, Perry A, Burger P, Ellison DW, Reifenberger G, von Deimling A, Aldape K, Brat D, Collins VP, Eberhart C, Figarella-Branger D, Fuller GN, Giangaspero F, Giannini C, Hawkins C, Kleihues P, Korshunov A, Kros JM, Beatriz Lopes M, Ng HK, Ohgaki H, Paulus W, Pietsch T, Rosenblum M, Rushing E, Soylemezoglu F, Wiestler O, Wesseling P; International Society of Neuropathology-Haarlem. International Society of Neuropathology-Haarlem consensus guidelines for nervous system tumor classification and grading. Brain Pathol 24:429-435, 2014
18. Massimino M, Antonelli M, Gandola L, Miceli R, Pollo B, Biassoni V, Schiavello E, Buttarelli FR, Spreafico F, Collini P, Giangaspero F: Histological variants of medulloblastoma are the most powerful clinical prognostic indicators. Pediatr Blood Cancer 60:210-216, 2013
19. Narin F, Bahadir S, Hanalioglu S, Karakaya D, Basar I, Isikay I, Soylemezoglu F, Akalan N, Bilginer B: Does gross total resection improve progression-free and overall survival in pediatric intracranial ependymomas? Single-center clinical experience of 61 cases. World Neurosurg 165:e469-e478, 2022
20. Northcott PA, Dubuc AM, Pfister S, Taylor MD: Molecular subgroups of medulloblastoma. Expert Rev Neurother 12:871-884, 2012
21. Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, Bouffet E, Clifford SC, Hawkins CE, French P, Rutka JT, Pfister S, Taylor MD: Medulloblastoma comprises four distinct molecular variants. J Clin Oncol 29):1408-1414, 2011
22. Northcott PA, Shih DJ, Remke M, Cho YJ, Kool M, Hawkins C, Eberhart CG, Dubuc A, Guettouche T, Cardentey Y, Bouffet E, Pomeroy SL, Marra M, Malkin D, Rutka JT, Korshunov A, Pfister S, Taylor MD: Rapid, reliable, and reproducible molecular sub-grouping of clinical medulloblastoma samples. Acta Neuropathol 123:615-626, 2012
23. Otte J, Dyberg C, Pepich A, Johnsen JI: MYCN function in neuroblastoma development. Front Oncol 10:624079, 2021
24. Pomeroy SL, Loeffler JS, Wen PY, Gajjar A, Eichler AF: Histopathology and molecular pathogenesis of medulloblastoma. UpToDate, edited by AF Eichler, UpToDate, Waltham, MA2015
25. Roussel MF, Robinson GW: Role of MYC in medulloblastoma. Cold Spring Harb Perspect Med 3:a014308, 2013
26. Schwalbe EC, Lindsey JC, Nakjang S, Crosier S, Smith AJ, Hicks D, Rafiee G, Hill RM, Iliasova A, Stone T, Pizer B, Michalski A, Joshi A, Wharton SB, Jacques TS, Bailey S, Williamson D, Clifford SC: Novel molecular subgroups for clinical classification and outcome prediction in childhood medulloblastoma: A cohort study. Lancet Oncol 18:958-971, 2017
27. Sharma T, Schwalbe EC, Williamson D, Sill M, Hovestadt V, Mynarek M, Rutkowski S, Robinson GW, Gajjar A, Cavalli F, Ramaswamy V, Taylor MD, Lindsey JC, Hill RM, Jäger N, Korshunov A, Hicks D, Bailey S, Kool M, Chavez L, Northcott PA, Pfister SM, Clifford SC: Second-generation molecular subgrouping of medulloblastoma: An international meta-analysis of Group 3 and Group 4 subtypes. Acta Neuropathol 138:309-326, 2019
28. Shih DJH, Northcott PA, Remke M, Korshunov A, Ramaswamy V, Kool M, Luu B, Yao Y, Wang X, Dubuc AM, Garzia L, John Peacock J, Mack SC, Wu X, Rolider A, Morrissy AS, Cavalli FMG, Jones DTW, Zitterbart K, Faria CC, Schüller U, Kren L, Kumabe T, Tominaga T, Ra YS, Garami M, Hauser P, Chan JA, Robinson S, Bognár L, Klekner A, Saad AG, Liau LM, Albrecht S, Fontebasso A, Cinalli G, De Antonellis P, Zollo M, Cooper MK, Thompson RC, Bailey S, Lindsey JC, Di Rocco C, Massimi JC, Michiels EMC, Scherer SW, Phillips JJ, Gupta N, Fan X, Muraszko KM, Vibhakar R, Eberhart CG, Fouladi M, Lach B, Jung S, Wechsler-Reya RJ, Fèvre-Montange M, Jouvet A, Jabado N, Pollack IF, Weiss WA, Lee JY, Cho BK, Kim SK, Wang KC, Leonard JR, Rubin JB, de Torres C, Lavarino C, Mora J, Cho YJ, Tabori U, Olson JM, Gajjar A, Packer RJ, Rutkowski S, Pomeroy SL, French PJ, Kloosterhof NK, Kros JM, Van Meir EG, Clifford SC, Bourdeaut F, Delattre O, Doz FF, Hawkins CE, Malkin D, Grajkowska WA, Perek-Polnik M, Bouffet E, Rutka JT, Pfister SM, Taylor MD: Cytogenetic prognostication within medulloblastoma subgroups. J Clin Oncol 32:886-896, 2014
29. Soylemezoglu F, Oz B, Egilmez R, Pekmezci M, Bozkurt S, Danyeli AE, Onguru O, Kulac I, Tihan T: Towards development of a standard terminology of the World Health Organization Classification of tumors of the central nervous system in the turkish language, and a perspective on the practical implications of the who classification for low and middle income countries. Turk Patoloji Derg 38:185-204, 2022