EUROPEAN JOURNAL OF CANCER 42 (2006) 2996–3003

available at www.sciencedirect.com

journal homepage: www.ejconline.com

Histopathological prognostic factors in medulloblastoma: High expression of survivin is related to unfavourable outcome ☆

C. Haberler[a,], I. Slavc[b], T. Czech[c], E. Gelpi[a], H. Heinzl[d], H. Budka[a], C. Urban[e], M. Scarpatetti[f], G. Ebetsberger-Dachs[g], C. Schindler[h], N. Jones[i], A. Klein-Franke[j], H. Maier[k], B. Jauk[l], A. Kiefer[m], J.A. Hainfellner[a]*

[a]*Institute of Neurology, Medical University of Vienna, Währinger Gürtel 18–20, A-1097 Vienna, Austria*
[b]*Department of Paediatrics, Medical University of Vienna, Vienna, Austria*
[c]*Department of Neurosurgery, Medical University of Vienna, Vienna, Austria*
[d]*Core Unit for Medical Statistics and Informatics, Medical University of Vienna, Vienna, Austria*
[e]*Division of Paediatric Haematology/Oncology, Department of Paediatrics and Adolescent Medicine, Medical University Graz, Graz, Austria*
[f]*Institute of Pathology, Medical University Graz, Graz, Austria*
[g]*Department of Paediatrics, Landesfrauen- und Kinderklinik, Linz, Austria*
[h]*Institute of Pathology, Landesnervenklinik Wagner Jauregg, Linz, Austria*
[i]*Landesklinik für Kinder- und Jugendheilkunde, Salzburg, Austria*
[j]*Department of Paediatrics, Medical University of Innsbruck, Innsbruck, Austria*
[k]*Institute of Pathology, Medical University of Innsbruck, Innsbruck, Austria*
[l]*Department of Paediatrics, LKH Klagenfurt, Klagenfurt, Austria*
[m]*Institute of Pathology, LKH Klagenfurt, Klagenfurt, Austria*

ARTICLE INFO

Article history:
Received 10 March 2006
Received in revised form
4 May 2006
Accepted 8 May 2006
Available online 25 September 2006

Keywords:
Medulloblastoma
Child
Prognostic markers
Histopathology
Survivin

ABSTRACT

Standard postoperative treatment of medulloblastoma consists of craniospinal irradiation and chemotherapy. Currently, only clinical factors are used for therapy stratification. To optimise treatment and patient outcome, biological prognostic markers are needed. In the present study we tested the prognostic influence of four histopathological parameters considered in recent publications as prognostic factors in medulloblastoma.We analysed a series of 82 Austrian medulloblastoma patients who were treated according to the consecutive HIT protocols for medulloblastoma conducted by the German Society of Paediatric Haematology and Oncology. Histological subtype and immunohistochemical expression of erbB-2, TRKC, and survivin were determined on paraffin embedded tumour tissue and correlated with patient outcome.Statistical analysis showed a significant correlation of high expression levels of survivin with decreased survival. None of the other investigated histopathological factors correlated significantly with patient outcome.Our data indicate that high survivin expression is related to unfavourable clinical outcome in medulloblastoma patients.

☆ A cooperative study of the Austrian Neurooncology Network (ANN).

* *Corresponding author:* Tel.: +43 1 40400 5504; fax: +43 1 40400 5511.
E-mail address: christine.haberler@meduniwien.ac.at (C. Haberler).

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2006.05.038

1. Introduction

Medulloblastoma is the most common malignant primary brain tumour in children. Therapy of medulloblastoma comprises maximal surgical resection of the tumour followed by craniospinal irradiation and chemotherapy. Such treatment causes long-term morbidity including endocrine and growth disturbances, as well as neurocognitive impairment, which is particularly severe in young children.[1]

Currently, patients are stratified into different therapy arms based on clinical parameters such as patient age, metastatic stage, and residual tumour size. These clinical prognostic parameters are used to distinguish between a group of high- and average- risk patients.[2] Yet, within the average risk group, clinical parameters do not identify a group of patients with a particular low risk of tumour recurrence, which could be treated with a substantially less toxic treatment regime as compared to standard treatment. Thus the major goal in the treatment of children with medulloblastoma is to identify patients who can be cured with a less toxic therapy while developing new treatment strategies for patients with a high risk of tumour recurrence. To this end biological markers are needed which (1) provide prognostic information and (2) serve as molecular targets for new treatment strategies.

Different prognostic parameters have been described in medulloblastoma (reviewed in Ref. [3]). In recent studies histopathological subtype,[4–6] erbB-2,[7] TRKC,[8] and survivin[9,10] have been reported as prognostic factors for patient outcome. However, so far none of these parameters are used in clinical management for therapy stratification. An essential prerequisite for translation of prognostic and predictive parameters into clinical practice is validation of their prognostic influence by independent investigators.

We analysed the prognostic impact of histopathological subtype, expression of erbB-2, TRKC, and survivin in a cohort of Austrian medulloblastoma patients operated on between 1990 and 2004 and treated according to consecutive HIT therapy protocols.

2. Materials and methods

2.1. Patients

Selection criteria for patients in this study were newly diagnosed medulloblastoma, operated on between 1990 and 2004 in Austria (Vienna, Graz, Linz, Salzburg, Innsbruck, and Klagenfurt); age at operation < 22 years, and randomisation into the consecutive multicentre trials (HIT) for medulloblastoma of the German Society of Paediatric Haematology and Oncology (GPOH).[11,12] Using these criteria, 126 patients were identified. No tumour tissue was available in 34 patients. Five tumours had other diagnoses at reclassification (four atypical teratoid/rhabdoid tumours and one ependymoma). In four patients no clinical data were available. One patient died one day after surgery and was therefore excluded from the study. Altogether a total of 82 patients with adequate tumour tissue and clinical follow-up data remained as study cohort.

Clinical characteristics are presented in Table 1.

Table 1 – Clinical characteristics

Factor	Distribution (%), $n = 82$
Age	
< 3years	11 (13.4)
⩾ 3years	71 (86.6)
Metastatic staging according to Chang[a]	
M0	59 (72.0)
M1	2 (2.4)
M2	5 (6.1)
M3	6 (7.3)
M2& 3	7 (8.5)
Residual tumour	
⩽ 1.5 cm^2	54 (65.9)
> 1.5 cm^2	28 (34.1)
Gender	
Female	27 (32.9)
Male	55 (67.1)

a In 3 patients no initial spinal MRI available.

Patient age at operation ranged from 0.76 to 21.6 years (median 7.3 years). Median follow-up time was 6.0 years (range from 0.51 to 14.4 years).

For assessment of the extent of surgical resection, CT or MRI scans were obtained within 72 h after surgery in all patients. For metastatic staging MRI of the spinal cord was performed before or after surgery in all but three patients. Cerebrospinal fluid cytology was analysed 2 weeks after surgery in a proportion of patients. Initial metastatic disease was classified according to Chang[13] as: M0 (no metastases), M1 (tumour cells within the cerebrospinal fluid), M2 (cerebral metastases), M3 (spinal metastases). The following treatment protocols were used:

(1) HIT'91 S (Sandwich-investigational arm I) consisted of ifosfamide, etoposide, high-dose methotrexate, cisplatin, and cytarabine given in two cycles before craniospinal irradiation (35.2 Gy total dose, 1.6 Gy/d plus boost to posterior fossa to 54 Gy).[11]
(2) HIT'91 E (Maintenance-standard arm II 'Philadelphia protocol') consisted of immediate postoperative craniospinal irradiation (35.2 Gy total dose, 1.6 Gy/d plus boost of 20 Gy to posterior fossa), with concomitant vincristine, followed by eight cycles of maintenance chemotherapy consisting of cisplatin, CCNU, and vincristine.[11]
(3) HIT 2000 consisted of immediate postoperative reduced conventional craniospinal irradiation (23.4, Gy, 1.6 Gy/d plus boost to posterior fossa to 54 Gy and 60 Gy to residual tumour) or hyperfractionated irradiation (36 Gy, 2×1 Gy/d plus boost to posterior fossa to 66 Gy and residual tumour 72 Gy) followed by HIT'91 E chemotherapy.
(4) HIT-SKK (92 baby protocol,[12] and (2000)) (patient age < 3 (or 4 years), respectively) consisted of cyclophosphamide, vincristine, high-dose methotrexate, carboplatin, and etoposide as well as intraventricular methotrexate.
(5) MET-HIT 2000 (initial metastatic disease) consisted of HIT-SKK plus hyperfractionated craniospinal irradiation (40 Gy, 2×1 Gy/d, plus boost to spinal metastases to 50 Gy

Table 2 – Patient categorisation into subgroups according to treatment protocols

Therapy protocol	Distribution (%), $n = 82$	M1-3[a] ($n = 20$)
HIT'91 S	18 (22.0)	5
HIT'91 E	29 (35.4)	5
HIT-SKK	14 (17.1)	4
HIT-2000	16 (19.5)	1[b]
MET-HIT 2000	5 (6.0)	5

a Metastatic stages according to Chang.[13]
b Initially classified as M0, but re-evaluated by reference center as M1.

and 60 Gy to posterior fossa, 66 Gy to tumour bed, and 72 Gy to residual tumour) followed by four courses of maintenance therapy.

Patient categorisation into subgroups according to treatment protocols is presented in Table 2.

The present study was approved by the Ethics Committee of the Medical University of Vienna.

2.2. Methods

For histopathological analysis, formalin fixed, routinely processed paraffin embedded tumour tissue was cut at 3 μm, and deparaffinised. Haematoxylin and eosin, Giemsa and Gomori reticulin stains were performed.

For immunohistochemical stainings primary antibodies were used to the following antigens at the indicated dilutions: synaptophysin (clone SY38, Dako, Glostrup, Denmark), 1:100; Ki-67 (clone MIB-1, Dako), 1:50; survivin (sc-10811, polyclonal, Santa Cruz Biotechnology, Inc, USA), 1:300; BAF47-INI protein (clone 25, BD Transduction Labs, San Diego, California, USA), 1:50; erbB-2 (clone CB11, Novocastra Laboratories Ltd, Newcastle, UK), 1:40; TRKC (sc-117, polyclonal, Santa Cruz Biotechnology), 1:200. For Ki-67, BAF47, erbB-2, and survivin heat-induced epitope retrieval was carried out in 0.01 M citrate buffer. For TRKC sections were pretreated with proteinase K 0.03%.

Detection of immunostaining was performed using the ChemMate kit (Dako) and diaminobenzidine as chromogene. Sections containing a breast carcinoma, foetal brain tissue, and glioblastoma were used as positive controls for erbB-2, TRKC, and survivin, respectively. For negative control the primary antibody was omitted.

2.3. Histopathology

Histopathologic classification of classic, nodular/desmoplastic (N/D), or large cell/anaplastic (LC/A) medulloblastoma subtype was based upon criteria described in the literature[4,6,14,15] and performed on a multi-headed microscope by two neuropathologists (CH and JAH) blinded to the clinical data. Tumour cells with angulated and moulded nuclei in areas of Homer Wright nuclear rosette formations or areas with prominent desmoplastic reaction due to leptomeningeal invasion were frequently detectable, but not considered as anaplastic subtype if further signs of anaplasia (nuclear wrapping, frequent mitoses and apoptoses) were lacking. N/D subtype was diagnosed if at least a single nodule with decreased cellularity and neuropil-like synaptophysin immunoreactive differentiation was detectable. To elucidate desmoplasia Gomori reticulin stain was used. Cases with intense desmoplastic reaction due to invasion into the leptomeninges but without nodule formation were not classified as N/D but as classic or LC/A subtype.

Mitotic frequency was assessed by counting mitotic figures in the Giemsa stain in the area with highest tumour cell proliferation, in a field sized 1 mm^2 that was defined by an ocular morphometric grid.

2.4. Immunohistochemistry

For the assessment of survivin and Ki-67 immunolabelling, 500 tumour cell nuclei were evaluated in each tumour specimen in fields showing the highest density of immunopositive nuclei. The fraction of labelled tumour cell nuclei was expressed as a percentage (survivin or Ki-67 index, respectively). To assess the expression of TRKC and erbB-2, tumours were divided into four groups: (1) 0; (2) <10%; (3) 10–50%, (4) >50% immunolabelled tumour cells.

To exclude atypical teratoid/rhabdoid tumours (AT/RT), we performed immunohistochemical analysis of the INI1 protein in all tumours.

2.5. Statistical analysis

The distributions of variables of interest were described either by absolute values and percentages or by median values and ranges.

Spearman rank correlation coefficient was used to assess associations between continuous variables. Fisher's exact test was used to assess group differences within dichotomous variables, and Mann–Whitney's U-test and Kruskal-Wallis test to assess group differences within continuous variables.

Overall survival time (OS) was defined as the period between the date of initial surgery and death of the patient. Survival times of patients still alive at the end of the observation period were considered censored. Progression free survival (PFS) was analogously defined as the time period between the date of initial surgery and tumour relapse (progression, recurrence or new occurrence of metastasis). Survival probabilities were calculated with the product limit method of Kaplan and Meier.[16] Univariate and multiple Cox proportional hazards regression models were used to assess the effects of variables of interest on OS and PFS.[17]

Survivin expression and mitotic frequency were converted to logarithmic values (base 2) before they were used in the Cox model in order to overcome potential problems caused by the skewed distributions of these variables. The variables TRKC and erbB-2 expression were used in the Cox model with their group score, that is, 1 for 0%, 2 for more than 0 until 10%, 3 for more than 10 until 50%, 4 for more than 50%. The use of a group score enables the detection of a monotone trend on survival in a flexible way.

All p values are results of two-sided tests. Values of $p < 0.05$ were considered statistically significant. As the main interest of the study was the prognostic evaluation of four factors (histological subtype, erbB-2, TRKC, and

survivin) we adjusted for multiple testing by applying a simple Bonferroni-type significance level of 0.0125. The results of all other statistical tests were considered exploratory rather than confirmatory so that no further adjustment for multiple testing was performed. The statistical software packages SAS (SAS Institute Inc., Cary, NC) and SPSS (SPSS Inc., Chicago, IL) were used for calculations.

3. Results

3.1. *Histopathology*

Nuclear immunohistochemical expression of INI protein was detectable in all tumours, thus cases of AT/RT mimicking morphological features of medulloblastoma were not included in the investigated sample.

3.1.1. *Histopathologic subtype*

Histopathological evaluation revealed 50 (61.0%) classic, 19 (23.2%) N/D, and 13 (15.8%) LC/A medulloblastomas (Fig. 1A, B and C). Clear-cut differentiation between classic and LC/A subtype was difficult in some cases. In 7/50 cases, which we considered as classic medulloblastomas, we found additionally small anaplastic foci.

3.1.2. *Immunohistochemical analysis*

Survivin (Fig. 1D) and Ki-67 immunoreactivity was observed in tumour cell nuclei. The expression pattern of TRKC (Fig. 1E)

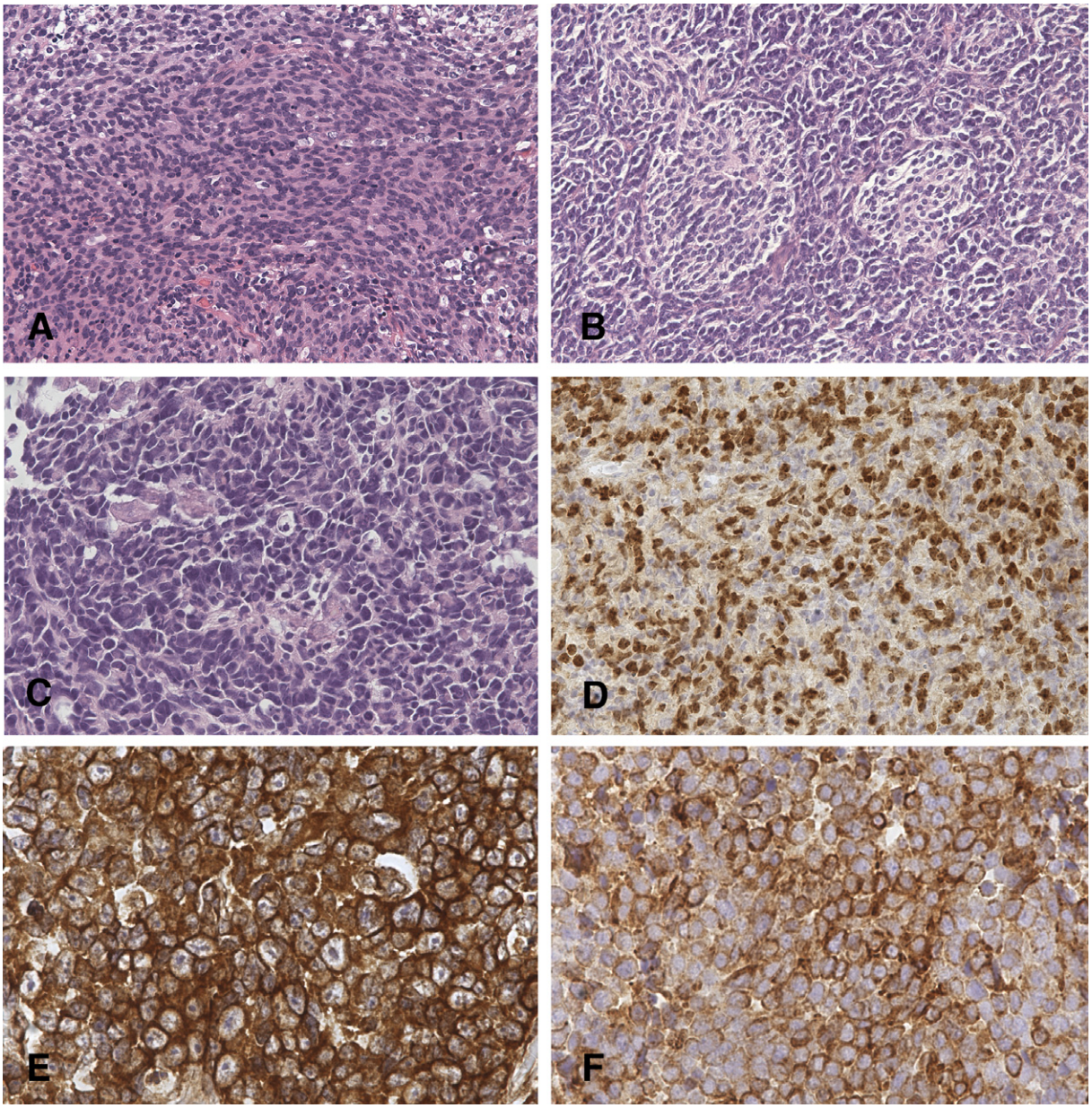

Fig. 1 – Histopathological medulloblastoma classification into classic (A), nodular/desmoplastic (B), and large cell/anaplastic (C) subtypes (A–C, haematoxylin and eosin; original magnification, × 200). High expression of survivin (D), TRKC (E), erbB-2 (F); (original magnification, D × 200; E, F × 400).

Table 3 – Results of immunohistochemical analysis

Factor	Distribution (%), $n = 82$
ErbB-2 expression	
0% tumour cells	33 (40.2)
< 10% tumour cells	25 (30.5)
10–50% tumour cells	12 (14.6)
⩾ 50% tumour cells	12 (14.6)
TRKC expression	
0% tumour cells	60 (73.2)
< 10% tumour cells	6 (7.3)
10–50% tumour cells	10 (12.2)
⩾ 50% tumour cells	6 (7.3)

and erbB-2 (Fig. 1F) appeared predominantly cytoplasmic, but due to the scant cytoplasm of tumour cells definite differentiation between cytoplasmic and membranous pattern was not possible. Results of immunohistochemical analysis of erbB-2 and TRKC expression are presented in Table 3. Survivin indices ranged between 4% and 63.8% (median: 16.7%). Distribution of survivin indices did not differ between the subgroups of patients treated with different therapy protocols ($p = 0.13$, Kruskal–Wallis test).

Mitotic frequency and Ki-67 proliferation index were assessed to analyse potential associations with survivin and histopathological subtype.

Mitotic frequency and Ki-67 proliferation indices ranged between 10 and 123/mm^2, and 3% and 97.8% (median: 36.5/mm^2, and 45.2%). The Spearman's correlation coefficient of survivin and Ki-67 proliferation index, survivin and mitotic frequency, and Ki-67 proliferation index and mitotic frequency was 0.61, 0.42, and 0.39, respectively (all $p < 0.001$).

In the N/D subtype survivin was predominantly expressed within the internodular, desmoplastic areas and was significantly higher in N/D compared to the classic subtype (Fisher's exact test, $p = 0.0032$ and Mann–Whitney U-test, $p = 0.029$).

Anaplastic medulloblastomas showed a significantly higher number of mitotic figures compared to classic medulloblastomas (Mann–Whitney U-Test, $p = 0.007$), whereas no significant differences between classic and N/D, and N/D and anaplastic tumours were detectable. Ki-67 proliferation index did not differ significantly between the subtypes.

3.2. *Survival analysis*

At last follow-up 60/82 (73.2%) patients were alive. In 25/82 (30.5%) of the patients, disease progression or recurrence occurred. Eighteen of 82 (22%) patients had died from progression of disease, 4/82 (4.9%) patients had died from other causes (sepsis: $n = 2$, central pontine myelinolysis: $n = 1$, chronic myelogenous leukemia: $n = 1$).

Table 4 – Log rank test and Cox regression analysis for progression free survival

Variable	Univariate		Multivariate Cox analysis	
	HR (95% CI)	P (Log-rank test)	HR (95% CI)	P (Wald test)
Age (<3/ ⩾ 3years)	0.28 (0.11–0.71)	0.0039	0.19 (0.07–0.51)	0.0009
M-staging (M0/M1–3)	2.71 (1.18–6.21)	0.014	1.98 (0.80–4.88)	0.14
Residual tumour size (⩽1.5/ > 1.5 cm^2)	2.02 (0.92–4.43)	0.07	2.20 (0.85–5.67)	0.10
Survivin ($\log_2$–transformed)[a]	1.65 (0.94–2.90)	0.08	1.93 (1.02–3.65)	0.042
Histopathological subtype		0.47	–	–
classic/nodular-desmoplastic	1.23 (0.51–2.99)			
classic/anaplastic	0.47 (0.11–2.06)			
ErbB-2 expression (0/ < 10%/10–50%/ > 50%)	1.03 (0.72–1.47)	1.00	–	–
TRKC expression (0/ < 10%/10–50%/ > 50%)	1.02 (0.66–1.58)	0.16	–	–

HR: hazard ratio, CI: confidence interval.
a The hazard ratios apply to a doubling of the survivin level.

Table 5 – Log rank test and Cox regression analysis for overall survival

Variable	Univariate		Multivariate Cox analysis	
	HR (95% CI)	P (Log-rank test)	HR (95% CI)	P (Wald test)
Age (<3/ ⩾ 3years)	0.26 (0.10–0.66)	0.0024	0.17 (0.06–0.47)	0.0006
M-staging (M0/M1–3)	2.20 (0.90–5.40)	0.08	1.92 (0.73–5.03)	0.19
Residual tumour size (⩽1.5/ > 1.5 cm^2)	1.61 (0.69–3.72)	0.26	2.25 (0.83–6.14)	0.11
Survivin ($\log_2$–transformed)[a]	2.59 (1.38–4.88)	0.0041	3.14 (1.50–6.59)	0.0024
Histopathological subtype		0.77	–	–
classic/nodular-desmoplastic	1.41 (0.53–3.70)			
classic/anaplastic	0.99 (0.28–3.47)			
ErbB-2 expression (0/ < 10%/10–50%/ > 50%)	0.96 (0.65–1.40)	0.88	–	–
TRKC expression (0/ < 10%/10–50%/ > 50%)	0.85 (0.51–1.42)	0.29	–	–

HR: hazard ratio, CI: confidence interval.
a The hazard ratios apply to a doubling of the survivin level.

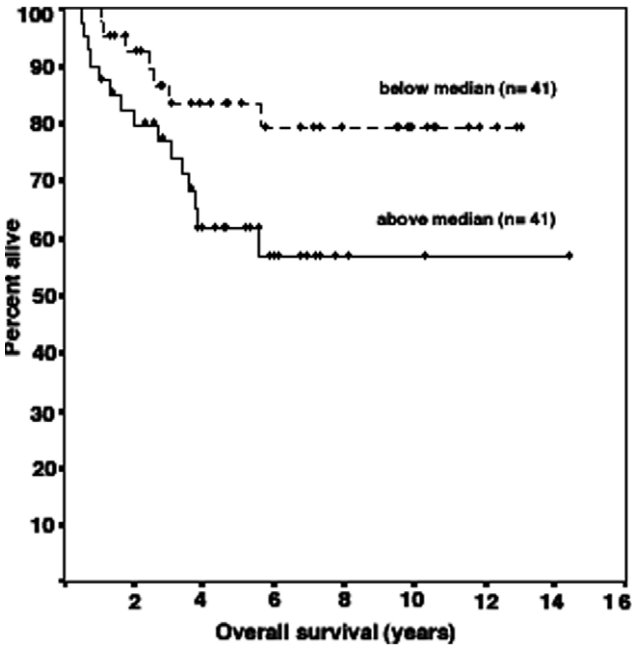

Fig. 2 – Kaplan Meier overall survival curve for medulloblastoma patients with survivin expression below and above the median (median: 16.7%, $p = 0.044$; Log rank test).

Overall survival (OS) at 5 and 10 years was 72% and 68%, respectively. 5- and 10-year progression free survival (PFS) was 68% and 64%, respectively.

Detailed results of statistical analysis of clinical and histopathological parameters are presented in Tables 4 and 5.

Among the histopathological factors, only survivin was associated significantly with patient survival in univariate (Log rank test) (Fig. 2) and multivariate (Cox regression) analysis, i.e. increasing survivin expression correlated with decrease in survival. These results still hold for OS after applying the Bonferroni-adjusted significance level of 0.0125. Ki-67 index and mitotic frequency did not gain significance for OS and PFS ($p > 0.05$).

When statistical analysis was repeated in a subset of patients older than 3 years and without initial metastatic disease ($n = 51$) treated according to HIT'91 and HIT'2000 protocols survivin expression retained statistical significance in univariate and multivariate Cox regression analysis for OS ($p = 0.017$ and 0.013, respectively), but did not reach significance for PFS ($p > 0.05$), probably due to the low number of events.

4. Discussion

In the present study we analysed the prognostic impact of four histopathological parameters on patient survival in a series of 82 medulloblastoma patients treated according to the consecutive HIT therapy protocols. Among the analysed factors, only high expression of survivin was related to poor outcome. Histopathological subtype, erbB-2, and TRKC expression in medulloblastoma have been analysed in several previous studies. However, contradictory results about the prognostic impact of these factors have been reported and so far none of these factors are used for therapy stratification.

The distinct morphological features of large cell medulloblastoma, and its association with aggressive clinical behavior, were first described by Giangaspero and co-workers.[15] This concept was extended in further studies showing that also significant anaplasia of tumour cells correlated with a dismal prognosis.[4–6,18,19] Lack of statistical significance in our series could be due to low statistical power, but anaplasia did not influence patient outcome in a larger series of 119 medulloblastoma patients[20] and two other series.[21,22] It has also been emphasised that the differences between classic and LC/A tumours are ambiguous,[6] thus subjective and prone to interobserver variability. Interestingly, one patient in our series with LC/A medulloblastoma remains in continuous complete remission at 12 years after diagnosis. In the cohort of Leonard also, one patient was alive 8 years after initial presentation.[19] Thus, there is evidence that LC/A morphology is not associated in all cases with aggressive clinical course.

TRKC is a receptor tyrosine kinase involved in the neurotrophin signalling pathway, which plays a key role in the regulation of growth, differentiation and death of neurons. Association of high expression levels of TRKC mRNA in medulloblastoma with favourable clinical outcome was first described by Segal and co-workers[23] and confirmed in a larger series.[8] However, in two independent studies with similar patient numbers[21,24] no prognostic significance for expression of TRKC mRNA could be detected. Immunohistochemical expression of TRKC was analysed in two studies so far,[20,25] but in both studies no significant impact of TRKC on patient outcome has been found. This lack of statistical significance could be due to differences between mRNA and protein expression levels of TRKC in medulloblastoma.

ErbB-2 is a member of the epidermal growth factor receptor (EGFR) family. EGFRs have been implicated in the development of many human cancers, and alterations of erbB-2 have been associated with more aggressive disease in several malignancies e.g. breast cancer.[26] In medulloblastoma, an association between high expression of erbB-2 and poor survival has been reported in several studies,[7,20,21,27] whereas no prognostic impact of erbB-2 has been reported by two other groups.[28,29] Although we tested erbB-2 immunopositivity with grade flexibility we could not detect a significant influence in our series, possibly due to low number of erbB-2 positive tumours and statistical low power.

Survivin is a new member of the family of inhibitors of apoptosis (IAP) and acts as inhibitor of apoptosis and regulator of mitosis.[30,31] Over-expression of survivin has been demonstrated in numerous types of cancer and has been proven as a marker of aggressive and unfavourable disease in cancer (reviewed in Ref. [32]). Based on the observation that survivin is expressed in malignancies but absent in most differentiated human tissues[30] therapeutic strategies to target survivin have been developed (reviewed in Ref. [32]).

In medulloblastoma, immunohistochemical expression of survivin has been correlated with clinical outcome only in two small patient cohorts ($n = 40$ and 42 patients) so far.[9,10] In both studies high expression of survivin was associated with unfavourable patient outcome. We confirm in our larger series the negative prognostic impact of survivin on patient survival in univariate and multivariate analysis. The significant prognostic impact of survivin was retained even

in a smaller cohort, including only patients older than 3 years and without initial metastatic disease. Thus survivin appears to be a robust prognostic factor in our series.

We detected a moderate correlation between survivin and Ki-67 expression as described previously in ependymoma.[33] As survivin is expressed only in G2 and M phases,[31] whereas Ki-67 is expressed in G1, S, G2, and M phases[34] lack of prognostic impact of Ki-67 index in contrast to survivin could be due to the function of survivin as inhibitor of apoptosis in addition to regulating mitosis. Previous *in vitro* and *in vivo* studies have demonstrated that expression of survivin increases chemo- and radioresistance.[35,36] Thus, the negative prognostic influence of high survivin expression in medulloblastomas could be due to an increased resistance to therapy-induced apoptosis in tumours highly expressing survivin.

Considering all these data, survivin might become a significant prognostic factor in medulloblastoma. Therapy stratification could be adjusted to the expression level of survivin (e.g. intensified adjuvant treatment in patients with high survivin expression). Furthermore, therapeutic targeting of survivin might be a new treatment option in medulloblastomas with high survivin levels. Yet, successful translation of survivin assessment into clinical practice depends on further studies confirming the prognostic influence of survivin expression in large patient series. If the prognostic significance of survivin can be confirmed, standardisation of laboratory methods for assessment of survivin expression and cut-off criteria for survivin hyper-expression have to be defined.

In conclusion, immunohistochemical expression of survivin seems to be a factor related to poor outcome in medulloblastoma patients.

Conflict of interest statement

None declared.

Acknowledgements

We thank Mrs E. Dirnberger, Mrs C. Karner, and Mrs H. Flicker for excellent technical assistance. Mrs G. Pammer, Mrs S. Schemel, Dr. K. Triebl, Dr. R. Prühlinger, Dr. A. Gamper, Dr. A. Gupper, and Dr. J. Trenkler for retrieval of clinical data and Dr. P. Pilz, Dr. A. Reiner-Concin, and Dr. W. Feichtinger for providing biopsy material.

REFERENCES

1. Radcliffe J, Packer RJ, Atkins TE, et al. Three- and four-year cognitive outcome in children with noncortical brain tumors treated with whole-brain radiotherapy. *Ann Neurol* 1992;**32**:551–4.
2. Zeltzer PM, Boyett JM, Finlay JL, 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**:832–45.
3. Rickert CH, Paulus W. Prognosis-related histomorphological and immunohistochemical markers in central nervous system tumors of childhood and adolescence. *Acta Neuropathol (Berl)* 2005;**109**:69–92.
4. Eberhart CG, Kepner JL, Goldthwaite PT, et al. Histopathologic grading of medulloblastomas: a Paediatric Oncology Group study. *Cancer* 2002;**94**:552–60.
5. Lamont JM, McManamy CS, Pearson AD, Clifford SC, Ellison DW. Combined histopathological and molecular cytogenetic stratification of medulloblastoma patients. *Clin Cancer Res* 2004;**10**:5482–93.
6. McManamy CS, Lamont JM, Taylor RE, et al. Morphophenotypic variation predicts clinical behavior in childhood non-desmoplastic medulloblastomas. *J Neuropathol Exp Neurol* 2003;**62**:627–32.
7. Gilbertson R, Wickramasinghe C, Hernan R, et al. Clinical and molecular stratification of disease risk in medulloblastoma. *Br J Cancer* 2001;**85**:705–12.
8. Grotzer MA, Janss AJ, Fung K, et al. TrkC expression predicts good clinical outcome in primitive neuroectodermal brain tumors. *J Clin Oncol* 2000;**18**:1027–35.
9. Fangusaro JR, Jiang Y, Holloway MP, et al. Survivin, Survivin-2B, and Survivin-deltaEx3 expression in medulloblastoma: biologic markers of tumour morphology and clinical outcome. *Br J Cancer* 2005;**92**:359–65.
10. Pizem J, Cort A, Zadravec-Zaletel L, Popovic M. Survivin is a negative prognostic marker in medulloblastoma. *Neuropathol Appl Neurobiol* 2005;**31**:422–8.
11. Kortmann RD, Kuhl J, Timmermann B, et al. Postoperative neoadjuvant chemotherapy before radiotherapy as compared to immediate radiotherapy followed by maintenance chemotherapy in the treatment of medulloblastoma in childhood: results of the German prospective randomized trial HIT '91. *Int J Radiat Oncol Biol Phys* 2000;**46**:269–79.
12. Rutkowski S, Bode U, Deinlein F, et al. Treatment of early childhood medulloblastoma by postoperative chemotherapy alone. *N Engl J Med* 2005;**352**:978–86.
13. Chang CH, Housepian EM, Herbert Jr C. An operative staging system and a megavoltage radiotherapeutic technic for cerebellar medulloblastomas. *Radiology* 1969;**93**:1351–9.
14. Giangaspero F, Bigner SH, Kleihues P, Pietsch T, Trojanowski QJ. Medulloblastoma. In: Kleihues P, Cavenee WK, editors. *World Health Organization classification of tumours. Pathology & genetics: Tumours of the nervous system*. Lyon: IARCPress; 2000. p. 129–37.
15. Giangaspero F, Rigobello L, Badiali M, et al. Large-cell medulloblastomas. A distinct variant with highly aggressive behavior. *Am J Surg Pathol* 1992;**16**:687–93.
16. Kaplan EL, Meier P. Non parametric estimation from incomplete observations. *Journal of the American Statistical Association* 1985;**53**:457–81.
17. Cox D. Regression models and life tables. *J Royal Stat Soc B* 1972;**34**:187–220.
18. Brown HG, Kepner JL, Perlman EJ, et al. Large cell/anaplastic medulloblastomas: a Paediatric Oncology Group study. *J Neuropathol Exp Neurol* 2000;**59**:857–65.
19. Leonard JR, Cai DX, Rivet DJ, et al. Large cell/anaplastic medulloblastomas and medullomyoblastomas: clinicopathological and genetic features. *J Neurosurg* 2001;**95**:82–8.
20. Ray A, Ho M, Ma J, et al. A clinicobiological model predicting survival in medulloblastoma. *Clin Cancer Res* 2004;**10**:7613–20.
21. Gajjar A, Hernan R, Kocak M, et al. Clinical, histopathologic, and molecular markers of prognosis: toward a new disease risk stratification system for medulloblastoma. *J Clin Oncol* 2004;**22**:984–93.

22. Giordana MT, D'Agostino C, Pollo B, et al. Anaplasia is rare and does not influence prognosis in adult medulloblastoma. *J Neuropathol Exp Neurol* 2005;**64**:869–74.
23. Segal RA, Goumnerova LC, Kwon YK, Stiles CD, Pomeroy SL. Expression of the neurotrophin receptor TrkC is linked to a favorable outcome in medulloblastoma. *Proc Natl Acad Sci U S A* 1994;**91**:12867–71.
24. Eberhart CG, Kratz J, Wang Y, et al. Histopathological and molecular prognostic markers in medulloblastoma: c-myc, N-myc, TrkC, and anaplasia. *J Neuropathol Exp Neurol* 2004;**63**:441–9.
25. Korshunov A, Savostikova M, Ozerov S. Immunohistochemical markers for prognosis of average-risk paediatric medulloblastomas. The effect of apoptotic index, TrkC, and c-myc expression. *J Neurooncol* 2002;**58**:271–9.
26. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;**235**:177–82.
27. Herms JW, Behnke J, Bergmann M, et al. Potential prognostic value of C-erbB-2 expression in medulloblastomas in very young children. *J Pediatr Hematol Oncol* 1997;**19**:510–5.
28. Korshunov A, Golanov A, Ozerov S, Sycheva R. Prognostic value of tumor-associated antigens immunoreactivity and apoptosis in medulloblastomas. An analysis of 73 cases. *Brain Tumor Pathol* 1999;**16**:37–44.
29. Nam DH, Wang KC, Kim YM, Chi JG, Kim SK, Cho BK. The effect of isochromosome 17q presence, proliferative and apoptotic indices, expression of c-erbB-2, bcl-2 and p53 proteins on the prognosis of medulloblastoma. *J Korean Med Sci* 2000;**15**:452–6.
30. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med* 1997;**3**:917–21.
31. Li F, Ambrosini G, Chu EY, et al. Control of apoptosis and mitotic spindle checkpoint by survivin. *Nature* 1998;**396**:580–4.
32. Altieri DC. Survivin, versatile modulation of cell division and apoptosis in cancer. *Oncogene* 2003;**22**:8581–9.
33. Preusser M, Wolfsberger S, Czech T, Slavc I, Budka H, Hainfellner JA. Survivin expression in intracranial ependymomas and its correlation with tumor cell proliferation and patient outcome. *Am J Clin Pathol* 2005;**124**:543–9.
34. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol* 1984;**133**:1710–5.
35. Chakravarti A, Zhai GG, Zhang M, et al. Survivin enhances radiation resistance in primary human glioblastoma cells via caspase-independent mechanisms. *Oncogene* 2004;**23**:7494–506.
36. Rodel F, Hoffmann J, Distel L, et al. Survivin as a radioresistance factor, and prognostic and therapeutic target for radiotherapy in rectal cancer. *Cancer Res* 2005;**65**:4881–7.