Histological Variants of Medulloblastoma Are the Most Powerful Clinical Prognostic Indicators

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INTRODUCTION

The World Health Organization’s classification defines four subtypes of medulloblastoma, that is, classic medulloblastoma, desmoplasic/nodular medulloblastoma, medulloblastoma with extensive nodularity (MBEN), and large-cell medulloblastoma [1]. In the 2007 WHO classification, the large-cell medulloblastoma category was expanded to include tumors showing highly anaplastic pleomorphic cells [2], and these two subtypes were divided into anaplastic and large cell ones, both presenting a worse clinical outcome than the other histotypes. Clinicopathological and biological studies have increasingly supported the hypothesis that medulloblastoma is a heterogeneous disease with different phenotypes and therapeutic outcomes. Histological classification in medulloblastoma has gained in importance and future treatment protocols will include histology as a risk factor. Reducing adjuvant therapy for patients with favorable histotypes can help to contain the long-term adverse effects of treatment, so identifying such patients would be important to enable therapy to be stratified. On the other hand, identifying patients at higher risk of progression enables their treatment to be intensified to hope fully improve their outcome [3–6]. We centrally reviewed all medulloblastoma cases from past 10 years reassessing their histology to ascertain its prognostic significance. Methods. Samples from 125 consecutive patients (99 males; 10 under age 3 years) were reviewed according to the two WHO classifications of 2000/2007. Results. Eighty-two patients did not have metastases, the primary tumor was completely resected in 101. The median follow-up was 96 months. Treatment was: our institutional protocol, that is, hyperfractionated accelerated radiotherapy (HART), for 39 non-metastatic cases up to 2003; according to the European PNET IV protocol in 31 cases; a HART-based strategy in 39 metastatic cases; tailored to the age below 3 years and based on high-dose chemotherapy in 10; and tailored to the patients conditions in 7. The 5-year PFS/OS rates were 76% and 81%, respectively. Histology was classic in 93 cases, nodular/desmoplasic in 20, anaplastic/large-cell in 9, and with extensive nodularity (MBEN) in 3. Stratification by residual disease after resection, metastases, age, or protocols was not prognostic. Histology suggested 5-year PFS rates of 82% for the desmoplastic and MBEN variants, 78% for classic medulloblastoma, 44% for the anaplastic/large-cell variants ($P = 0.01$). Multivariable analysis demonstrated statistically significant difference in PFS by histology ($P = 0.02$), due to the poor prognosis of anaplastic/large-cell medulloblastoma. Conclusions. Tailoring treatments to known risk factors cancelled all prognostic differences, except for anaplasia (not considered as such within previous trials) which proved the most powerful prognostic factor, warranting appropriate treatment intensification. 

Non-Metastatic Tumor Treatments 1998–2003 [Non-M Hyperfractionated Accelerated Radiotherapy (HART)]

During this early period, patients received postoperative vincristine (1.4 mg/ m2 iv) plus methotrexate (8 g/m2, 6-hour infusion), etoposide (2.4 g/m2, 10-hour infusion), cyclophosphamide

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(4 g/m², five 1-hour infusions), and carboplatin (0.8 g/m²), given during a 2-month schedule. Then HART was delivered to the neuraxis at a total dose of 39 Gy (1.3 Gy/fraction, 2 fractions/day) for children over 10 years old at the time of their diagnosis or a total dose of 20.8–26 Gy for those under 10 years old, with a boost to the tumor bed of up to 60 Gy (1.5 Gy/fraction, 2 fractions/day) whatever the patient’s age. Four weeks after completing radiotherapy, a maintenance therapy with vincristine and lomustine was given for 1 year.
Non-Metastatic Tumor Treatment 2003–2008 (PNET-IV)

Up until 2006, patients were enrolled in the randomized SIOP-PNET IV trials comparing postoperative hyperfractionated radiotherapy (1.0 Gy twice daily with an 8-hour interval between fractions, a total neuraxis dose of 36 Gy, a whole posterior fossa dose of 60 Gy, and a boost of up to 68 Gy to the tumor bed) with conventional/standard radiotherapy (23.4 Gy to the neuraxis and 54 Gy to the whole posterior fossa), followed by the same chemotherapy regimen consisting of eight cycles of cisplatin, lomustine, and vincristine. During RT, up to eight doses of vincristine 1.5 mg/m² (maximum 2 mg) were given once a week in both treatment arms. Adjuvant chemotherapy started 6 weeks after completing RT. Eight cycles of cisplatin 70 mg/m² IV, lomustine 75 mg/m² orally on day 1, and vincristine 1.5 mg/m² IV on days 1, 8, and 15 were administered, with 6-week intervals between cycles. After the trial ended in December 2006, the preliminary results prompted the recommendation that patients continued to be treated according to the standard radiotherapy arm, omitting the weekly vincristine during RT.

Metastatic Tumors (mHART)

The treatment for these patients has been reported elsewhere [8]; it was based on an intensive postoperative induction treatment with high-dose methotrexate plus vincristine, high-dose etoposide, high-dose cyclophosphamide, and high-dose carboplatin, as in the above-described schedule for non-metastatic cases (1998–2003). Afterwards, patients received HART to the neuraxis with total craniospinal doses of 31 Gy for patients under 10 years old at diagnosis in complete remission at all tumor sites before HART, and 39 Gy for all the other cases. Boost to the tumor bed was given in two daily fractions of 1.5 Gy, up to 59.7 Gy if the total dose to the neuraxis was 31 Gy, or up to 60 Gy if it was 39 Gy. After HART, children could receive either maintenance treatment with vincristine and lomustine for 1 year if they had been in complete remission prior to HART, or two consolidation myeloablative courses with high-dose thiotepa (900 mg/m² in 3 days followed by autologous hematopoietic stem cell reinfusion) in all the other cases.

Treatment in Cases Under 3 Years of Age (Young Children)

This strategy included an induction regimen identical to those used for older children before 2006 and for metastatic cases, followed by two courses of myeloablative chemotherapy (tandem thiotepa at a dose of 900 mg/m² IV over 3 days) followed by local RT (54 Gy, standard fractionation) or craniospinal RT (23.4 Gy to the neuraxis, standard fractionation) in patients with residual or metastatic disease not reaching complete remission after induction chemotherapy. Treatment protocols received Ethical Committees approval and parents consent was obtained before treatment.

Other Treatments

Three children with a very poor performance status after surgery were given a tailored treatment consisting of two courses of cisplatin and etoposide, administered postoperatively, followed by RT to the neuraxis for a total dose of 23.4 Gy with a posterior fossa boost of 54 Gy, and then another four courses of cisplatin and etoposide. Four patients over 18 years of age received craniospinal irradiation for a total dose of 36 Gy with a posterior fossa boost of 54 Gy, followed by a maintenance treatment with oral lomustine and IV vincristine for 1 year.

Statistical Methods

The association between categorical variables was tested using the Chi-square test. Overall survival (OS) and progression-free survival (PFS) were estimated with the Kaplan–Meier method; between group comparisons were performed using the log-rank test. For OS, time was defined as the interval from the date of starting chemotherapy to the date of death for all causes, with censoring at the date of latest follow-up visit for alive patients. For PFS, time was the interval from the date of starting chemotherapy to the date of progression or death, whichever occurred first, with censoring at the latest follow-up visit for alive and progression-free patients. To analyze the prognostic effect of tumor histology (classic, desmoplastic, anaplastic/large cell) while adjusting for residual tumor (presence/absence) and treatment type (non-mHART, PNET IV, mHART, young children, other) we fitted multivariable Cox regression models [9]. Results are reported in terms of hazard ratio (HR), the corresponding 95% confidence interval and two-sided Wald test P-value. Confidence intervals not including the value of one indicate a significant difference toward the reference category. To control for overfitting due to the model high dimensionality as compared with the low number of events the linear predictor of a multivariable Cox model including residual tumor and treatment type was included as adjusting score, together with tumor histology, in a further Cox model. In the latter, we applied penalized maximum likelihood estimation methods [10]. The significance level was set at 5%. All statistical analyses were performed using SAS (SAS Institute, Inc., Campus Drive, Cary, North Carolina 27513) and R (http://www.r-project.org) software; in particular, penalized maximum likelihood estimation was performed using the R package penalized.

Histological Review

All histological samples were reviewed up front by two of the authors (BP and FG) based on the two WHO classifications of 2000 [1] and 2007 [2]. Samples from patients diagnosed before 2000 were re-reviewed for the purposes of this report. Since anaplastic medulloblastoma and the large-cell variant were included in the same subgroup in the WHO 2000 classification, they were kept as a single subgroup even after the 2007 classification separated them into two different subgroups. The general criteria used to define anaplasia were similar to those reported by Eberhart et al. [11], that is, increased nuclear size with marked pleomorphism widespread in different areas. Neoplastic cells with large and round nuclei, prominent nucleoli and a variable amount of eosinophilic cytoplasm can be seen, and are more common in anaplastic variant. Neoplastic cells are often enlarged three time the size of a red blood cell. Numerous mitoses are often atypical. Large clusters of apoptotic cells and necrosis were often seen. Cell wrapping and nuclear molding were identified in almost all tumors. These features have to be diffuse; finding them in focal areas is not enough [6]. A feature to not overlook is the presence of neoplastic cell discohesion.
RESULTS

The 125 patients enrolled from February 1998 to September 2008 (99 males), were a median age of 8 years (range 20 months to 39 years), and 20 patients were over 18 years old. Postoperative staging documented residual tumors in 24 patients (over 1.5 cm² in 15) cases and metastases (M1-M4) in 43, 16 of them presenting also residues. Table I summarizes the clinical features of all the patients diagnosed and treated before 2008 and Table II lists those with anaplastic/large cell tumors treated after 2008.

Treatment

Based on the patients’ stage and the protocols in use at the time, the non-mHART treatment was administered to 39 patients, the PNET IV to 31, the mHART to 38, the young children treatment to 10, and the above-explained other treatments to 7 patients.

Histology

Ninety-three samples were classified as classic medulloblastoma, 20 as desmoplastic/nodular type, 9 as anaplastic/large-cell, and 3 as MBEN. Three of the 9 anaplastic/large cell tumors were classified as large cell subtype, but some islets of large cells were identified also in 2 other predominantly anaplastic tumors.

Outcome

After a median follow-up of 98 months (interquartile range 60–125 months), the PFS was 76.4% (95% confidence interval: 69.1, 84.5%) at both 5 and 10 years, and the OS was 80.9% (74.2, 88.3%) at 5 years and 73.4% (65.3, 83.5%) at 10 years. Relapses were recorded a median 23 months (range 1–48) after diagnosis in 28 cases. They were local in 14 patients, disseminated in 13, and both local and disseminated in 1. Patients with anaplastic tumors had 5/5 local relapses a median 12 months after their diagnosis (mean 14 months).

Gender, metastases, therapeutic protocol, and age at diagnoses had no prognostic impact. Patients with anaplastic tumors had metastases in four cases, and residual disease in 1. One child was under 3 years of age at the time of diagnosis; these features were not statistically different from those of the group of patients as a whole.

The PFS for the 24 patients who began adjuvant treatment with residual posterior fossa disease was 65.8% (49.9, 86.8%) at both 5 and 10 years, while it was 79.4% (71.6, 88.1%) at 5 and 10 years for the 101 cases without residual disease (P = 0.09). The OS for the cases with residual disease was 67.9% (52.6, 87.6%) at 5 years and 63.0% (47.0, 84.5%) at 10 years. It was 84.6% (77.5, 92.4%) at 5 years and 76.3% (67.2, 86.7%) at 10 years for the patients with completely resected tumors (P = 0.054). When patients were divided into three subgroups: patients with no residual tumor or metastases (n = 77), patients with metastases (n = 49), and those with residual tumor but no metastases (n = 8), no difference emerged in their PFS and OS rates.

As regards the histological diagnoses, we included MBEN in the desmoplastic/nodular subgroup for the purposes of statistical analysis because there were only three cases of MBEN in our sample. The PFS and OS curves differed significantly by histotype (P = 0.010 and 0.019, respectively; Figs. 2 and 3). For the 93 patients with classic medulloblastoma, the PFS was 78.1% (69.8, 87.3%) at both 5 and 10 years, and the OS was 81.0% (73.2, 89.6%) and 76.3% (67.5, 86.1%) at 5 and 10 years, respectively. For the 20 patients with desmoplastic/nodular medulloblastoma or the three with MBEN, the PFS was 82.4% (68.1, 99.7%) at 5 and 10 years, and the OS was 91.3% (80.5, 100.0%) and 79.5% (63.1, 100.0%), respectively. One of the three children with MBEN, who had residual tumor after resection and metastases at diagnosis, had a disease relapse after 17 months and died of his disease after 24 months. For the nine patients with anaplastic/large-cell medulloblastoma, the PFS was 44.4% (21.4, 92.3%) at both 5 and 10 years, and the OS was, respectively, 53.3% (28.2, 100.0%) and 35.6% (12.8, 98.9%) at the two time points. Multivariable Cox analysis showed that histotype significantly influenced the PFS (P = 0.022), mainly because cases of anaplastic/large cell medulloblastoma had a worse prognosis than those with the other subtypes. The corresponding HR versus desmoplastic/nodular medulloblastoma was 5.00 (95% confidence interval: 1.43–17.86). In terms of PFS the prognosis for classic medulloblastoma did not differ significantly from that of desmoplastic/nodular medulloblastoma (HR = 1.43; 0.51–3.97). As for OS, HR vs.

![Table I. Clinical Features and Outcome of Patients Treated Before 2008](image_url)

**TABLE I. Clinical Features and Outcome of Patients Treated Before 2008**

<table>
<thead>
<tr>
<th>All histotypes</th>
<th>Classic (with residue)</th>
<th>Desmoplastic (with residue)</th>
<th>Anaplastic/large cell (with residue)</th>
<th>Treatment (n)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over 3 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic</td>
<td>32 (15)</td>
<td>3 (1 MBEN)</td>
<td>4 (1)</td>
<td>mHART (4)&lt;sup&gt;b&lt;/sup&gt; (2/4 +MAT)</td>
<td>2 CCR, 2 DOD (1/2 CCR)</td>
</tr>
<tr>
<td>Residue only</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-meta/non residue</td>
<td>52</td>
<td>15</td>
<td>4</td>
<td>HART non m (3); PNET IV (1)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 CCR, 2 DOD; 1 DOD</td>
</tr>
<tr>
<td>Below 3 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic</td>
<td>2 (1)</td>
<td>1 MBEN</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residue only</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-meta/non-residue</td>
<td>3</td>
<td>1 MBEN</td>
<td>1</td>
<td>Infants, with RT (1)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 DOD</td>
</tr>
</tbody>
</table>

MAT, myeloablative therapy; CCR, continuous complete remission; DOD, dead of disease; RT, radiotherapy. *1/8 < 10 years. <sup>b</sup>One large cell.

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desmoplastic/nodular medulloblastoma was 3.49 (1.10–11.12) for anaplastic/large-cell medulloblastoma and 1.46 (0.57–3.77) for classic medulloblastoma ($P = 0.091$).

Outcome for anaplastic medulloblastoma patients after 2008. In the light of these results, treatment was intensified for patients with the anaplastic/large-cell histotype, adopting the strategy used for metastatic medulloblastoma and consolidating with two myeloablative courses after HART, irrespective of any presence of metastases. From 2008 to 2011, we treated seven patients in this way, four with metastatic disease at diagnosis (two of them with residual disease as well), one under 3 years old at the time of diagnosis and $1/7$ with the large-cell subtype. These aspects did not differ statistically from the above reported series as a whole, or from the anaplastic subgroup. With a median follow-up of 27 months (range 8–38), six of these seven patients remained in complete remission while one boy relapsed with skeletal dissemination 12 months after diagnosis, having obtained a complete remission of residual tumor and metastases by the end of the treatment.

The 2/4 patients treated before 2008 according to the mHART protocol including the post-radiation myeloablative courses, one was alive free of disease at 59 months after diagnosis while the second had a local relapse after 12 months and died. The 3-year PFS and OS of these nine patients were, respectively, 71% (56, 88%) and 69% (50, 88%).

**DISCUSSION**

Apart from clinical staging based on the Chang system [7], which mainly uses the presence of overt metastatic disease and the degree of residual tumor after resection [12], subsequent papers dealing with the prognosis for childhood medulloblastoma have indicated that histological classification has a significant prognostic impact. Two rare subtypes lie at the opposite ends of the histological spectrum, that is, MBEN and anaplastic/large-cell medulloblastoma, respectively, associated with the best and worst clinical outcomes [13,14]. The WHO classification of 2007 further separated the anaplastic and large-cell medulloblastoma histotypes, though both carry a poor prognosis [2,15]. For the purposes of this article, our review of the slides was based on criteria for classifying anaplasia as suggested by other authors [6,11,16], and the large-cell and anaplastic variants of medulloblastoma were combined into a single category. In actual fact, foci of anaplasia are seen in tumors mainly revealing the features of classic medulloblastoma and, more rarely, in desmoplastic cases too, but the definition of anaplastic medulloblastoma was only used when the anaplastic phenotype was widespread. This meant that only 7% or our series was classified as anaplastic, a proportion similar to the one described by Ellison et al. among the 207 patients considered in the PNET3 trial [17], and by Eberhart et al. [11] in the Pediatric Oncology Group series. If we exclude the 20 patients over 18 years old at diagnosis (only one of whom had anaplastic medulloblastoma) from our series, the ratio remains

<table>
<thead>
<tr>
<th>Anaplastic/large cell (with residue)*</th>
<th>Treatment (n)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over 3 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic</td>
<td>3 (1)</td>
<td>mHART (3) all MAT</td>
</tr>
<tr>
<td>Residue only</td>
<td>1</td>
<td>mHART (1) with MAT</td>
</tr>
<tr>
<td>Non-meta/non residue</td>
<td>2</td>
<td>mHART (2) all MAT</td>
</tr>
<tr>
<td>Below 3 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic</td>
<td>1 (1)</td>
<td>Infants, with RT (1)</td>
</tr>
<tr>
<td>Residue only</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Non-meta/non-residue</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

MAT, myeloablative therapy; CCR, continuous complete remission; DOD, dead of disease; RT, radiotherapy. *6/6 < 10 years.

![Fig. 2. PFS according to histotype.](Pediatr Blood Cancer DOI 10.1002/pbc)  
![Fig. 3. OS according to histotype.](Pediatr Blood Cancer DOI 10.1002/pbc)
for the purposes of stratifying treatments [23,24]. Our data indicate that, irrespective of the subjective assessment of anaplasia, histology alone can distinguish between two groups with a significantly different prognosis. Even more importantly, assessing anaplasia was not a surrogate for clinical staging, it even proved a better predictor of outcome than metastatic extension (which was no longer significant after tailored treatment). Another finding relates to the characteristic pattern of recurrence that, unlike the majority of medulloblastomas, was exclusively local, emphasizing its particularly aggressive nature [25].

Previous reports on the prognostic impact of histology in this setting never arrived at the conclusion that this could be the sole prognostic factor, probably because the protocols adopted in the other series relied on less intense treatments and consequently achieved less favorable results for subgroups known to have poor prognosis, such as children with metastases, residues and/or those up to 3 years old. Our results are also the first to show better preliminary results (after a follow-up longer than 2 years) in a homogeneously and prospectively treated series of children with large cell/anaplastic medulloblastoma.

We conclude that irrespective of clinical stage, anaplasia should be included among the negative prognostic variables, and patients with this histotype (diagnosed at pathology by dedicated experts) deserve the most intensive treatment available. An intensified treatment, such as the one adopted in our cases with metastases [6], proved capable of controlling progression of the disease in the majority of cases.

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REFERENCES