RELATIONSHIP BETWEEN CLINICAL FEATURES, GENETIC FACTORS, AND PROGNOSIS IN NEOPLASTOMA PATIENTS: A SINGLE INSTITUTION'S EXPERIENCE

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Abstract — Between 1997 and 2003, 47 patients with neuroblastoma were treated at the Mother and Child Health Institute of Serbia. The objective of the present study was to determine the relationship between clinical and genetic features and prognosis in neuroblastoma.

The 5-year survival rate was best in the age group of less than 1 year (73% vs. 15%; p=0.007) and significantly worse for patients with stage IV than for “non stage IV” patients (13% vs. 47%; p= 0.015).

The difference in 5-year survival between the 1p deletion positive and 1p deletion negative groups was statistically significant (p= 0.008).

Overall 5-year survival was significantly worse for patients with N–myc amplification (19% vs. 58%; p= 0.014).

The difference in the 5-year survival for male and female patients was not statistically significant (p>0.05). Our report demonstrates that N–myc amplification, 1p deletion, stage IV, and age of 1 year and over are factors pointing strongly to an unfavorable outcome in neuroblastoma patients.

INTRODUCTION

Neuroblastoma (NB), a tumor derived from the neural crest, is the most frequent extra cranial solid tumor in childhood. It is extremely heterogeneous in terms of biological, morphological, and clinical characteristics.

The prognosis of neuroblastoma varies considerably with many factors: sex, age at diagnosis, clinical stage, primary site, and treatment protocol (Jaffe et al., 1976; Brodeur et al., 1993). Further prognostic factors identified include increased levels of vanillylmandelic acid (VMA), the homovanillic acid ratio (HVA), and several serological factors (neuron–specific enolase — NSE, ferritin, and lactate dehydrogenase –LDH) (Graham–Pole et al., 1983; La Brosse et al., 1980). Recently, several genetic abnormalities have been identified in advanced stages of NB: diploidy or tetraploidy (Look et al., 1991), deletions at the distal short arm of chromosome 1 (loss of heterozygosity — LOH) (Brodeur et al., 1977; Fong et al., 1989), and amplification of the N–myc oncogene (Brodeur et al., 1984).

In the present retrospective study, we report on a single institution’s experience with neuroblastosomas over a 5-year period by describing the relationship between clinical features, genetic factors, and prognosis in these patients.

PATIENTS AND METHODS

Patients

Between January 1997 and June 2003, 53 patients with neuroblastoma were diagnosed at the Mother and Child Health Institute of Serbia (MCIS).

The objective of the present retrospective study was to determine the relationship between clinical and genetic features and prognosis in neuroblastoma. The disease staging was classified according to the International Neuroblastoma Staging System (INSS) (Brodeur et al., 1993). The histopathologic diagnosis was established according to the Shimada classification (Shimada et al., 1984; Shimada et al., 1999). Prognostic factors analyzed
in our study included clinical (sex, age at diagnosis, and the clinical stage of NB) and genetic (N–myc amplification — NMA and 1p deletion) features.

Patients were also divided into two age groups: children under 1 year of age and children over 1 year of age. Patients were excluded from the study if the initial diagnosis or treatment was not made at the MCIS and if survival information was lacking.

Methods

Genetic analysis

Tumor material was taken either from the primary tumor after resection or biopsy, or from bone marrow with sufficient tumor cell infiltration for analysis.

Deletion of the short arm of chromosome 1 and N–myc amplification were analyzed by different techniques: FISH, PCR, and additional cytogenetic analysis in some cases (del 1p36, double minute chromosomes — dmin, and homogeneously staining regions — HSRs).

FISH analyses

To determine the integrity of 1p, a double–target in situ hybridization was performed with centromere D1Z1 (CytoCell) and telomere D1Z2 (CytoCell) specific probes, using the protocol recommended by CytoCell. The N–myc copy number was determined using the 2p24/D2Z probe (Q–biogene).

Cytogenetic analysis

For cytogenetic analyses, cells from bone marrow were cultured for 24 hours and then treated with colcemid, harvested, and fixed according to routine procedures. Karyotypes were described according to international nomenclature (ISCN, 1995).

Molecular analyses

High–molecular–weight DNA was isolated from the tumor and from peripheral blood lymphocytes using standard procedures according to Miller et al. (1988). Determination of LOH 1p was performed by analysis of paired constitutional and tumor DNA as a template for PCR amplification of two VNTR sequences, D1S80 and D1S76 as described previously (Peter et al., 1992).

Statistical analysis

Comparisons between proportions were performed using either the chi square likelihood ratio test or Fisher’s exact test (FET). Student’s two–sample t test was used to compare the means of two independent samples, while the Mann–Whitney U test (nonparametric method) was used to compare medians. Overall survival (OS) time (5 years) was defined as the time from diagnosis to death regardless of the cause (relapse, disease progression, therapy–related, or other causes) or to the date of last observation in surviving patients. Estimates of OS±SE were calculated

<table>
<thead>
<tr>
<th>Table 1. Patient Characteristics</th>
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<tbody>
<tr>
<td><strong>Characteristics</strong></td>
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<tr>
<td></td>
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<tr>
<td>Total</td>
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<td>IVs</td>
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<tr>
<td>Histology</td>
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<tr>
<td>NB</td>
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<td>GN</td>
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</table>

Note: p* — p value for log rank test; mo — months; NB — neuroblastoma; GN — ganglioneuroblastoma
according to the Kaplan and Meier product-limit method. Comparisons of estimated survival curves were performed by means of the log-rank test. Association between prognostic factors was tested by examining the Pearson correlation coefficient and by the Mantel-Haenszel chi-square test of trend using contingency tables.

All results are shown for the hazard ratio (HR) and 95% confidence interval (CI) for HR. The reported p values are all two-sided with 95% probability. Microsoft Excel software was used for data management, while SPSS statistical software was used for statistical analyses.

RESULTS

Patients

Among the 53 patients with neuroblastoma registered in the database between January 1997 and June 2003, six patients were excluded from the study for the following reasons: initial diagnosis not made at MCIS (two patients), treatment and follow-up not provided at MCIS (one), and lack of survival information (three); a total of 47 patients were included in the analyses.

Sex

There were 34 male and 13 female patients (2.62:1 ratio). The 5-year survival rate for the whole study period was 23% for male and 37% for female patients and the difference was not statistically significant (p > 0.05) (Table 1).

Age at diagnosis

The median age at diagnosis for all patients was 37 months (range 0.5–190 months) (Table 1), with 83% diagnosed before the age of 6 years. Survival by age at diagnosis is shown in Figure 1. Age at diagnosis was grouped into the following: less than 1 year of age and 1 year of age or more. There were 12 (25%) children under the age of 1 year. The 5-year survival rate was better in the age group of less than one year than in older children (73% vs. 15%). The difference in survival between the two age groups was statistically significant (p = 0.007) (Table 2).

Clinical stage

Patients were staged according to INSS; the distribution was as follows: stage I and II (n = 7), stage III (n = 13), stage IV (n = 26), and stage IVs (n = 1) (Table 1). Survival by clinical stage is shown in Figure 2. Stage I, II, III, and IVs patients were combined as “non-stage IV” because of the similar survival rates. The 5-year survival rate was significantly worse for patients with stage IV than for “non stage IV” patients (13% vs. 47%; p = 0.015) (Table 2).

![Survival Functions](image.png)

Fig. 1. Survival of neuroblastoma patients by age.
Genetic characteristics of tumors

Chromosome 1p36.3 integrity and N–myc status were not routinely investigated over the entire study period.

Chromosome 1p36.3 analyses

Twenty three chromosome 1p36.3 investigations using the FISH, PCR, or cytogenetic method were performed; 24 were not assessable.

Table 2. Five–year overall survival; unadjusted hazard ratio in relation to prognostic factors (univariate analysis)

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Total</th>
<th>Death</th>
<th>OS</th>
<th>SE OS</th>
<th>p</th>
<th>HR</th>
<th>95% CI</th>
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<td></td>
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<tr>
<td>&lt; 1 Year</td>
<td>12</td>
<td>3</td>
<td>73%</td>
<td>0.13</td>
<td>0.007</td>
<td>1.00</td>
<td></td>
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<tr>
<td>≥1 Year</td>
<td>35</td>
<td>29</td>
<td>15%</td>
<td>0.06</td>
<td>2.27</td>
<td>1.21–4.25</td>
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</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Female</td>
<td>14</td>
<td>8</td>
<td>37%</td>
<td>0.16</td>
<td>0.157</td>
<td>1.25</td>
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<td>33</td>
<td>24</td>
<td>23%</td>
<td>0.08</td>
<td>1.00</td>
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<td>Non-stage IV</td>
<td>21</td>
<td>10</td>
<td>47%</td>
<td>0.12</td>
<td>0.015</td>
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<td>Stage IV</td>
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<td>22</td>
<td>13%</td>
<td>0.07</td>
<td>2.58</td>
<td>1.08–6.16</td>
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<td>0.014</td>
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<td>0.17</td>
<td>6.92</td>
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<td>13</td>
<td>4</td>
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<tr>
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<td>10</td>
<td>9</td>
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<td>0.12</td>
<td>4.80</td>
<td>0.67–34.63</td>
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<tr>
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</tbody>
</table>

Note: OS — overall survival; SEOS — standard error; p  — p value for log rank test; HR — hazard ratio; CI — confidence interval; NMA — N–myc amplification

Fig. 2. Survival of neuroblastoma patients by stage.
Thirteen tumors (56%, 5%) showed no 1p36.3 aberrations; 10 tumors (43, 4%) exhibited 1p36.3 deletion. The difference in 5-year survival between the 1p36.3-positive and 1p36.3-negative group was statistically significant (p = 0.008); the lower 95% CI may be related to a smaller number of patients in each group (Table 2; Fig. 3).

**NMA analyses**

Analyses of NMA were performed on 27 tumors by FISH; 20 were not assessable. Seven (25.9%) of 27 NB tumors were with amplified N-myc (Table 2). The 5-year survival was significantly worse for patients with NMA (19% vs. 58%; p = 0.014) (Table 2; Fig. 4).

**Univariate Analysis by OS Rates**

The results of univariate analysis and hazard ratio in relation to prognostic factors are summarized in Table 2 and demonstrate a significantly inferior prognosis for NMA, 1p deletion, stage IV, and age of 1 year and over at diagnosis.

**DISCUSSION**

In 47 children with neuroblastoma diagnosed and treated in a single institution, the impact of clinical (sex, age and stage) and genetic (1p deletion and NMA) parameters on overall survival was investigated.

Univariate analysis revealed that age at diagnosis was a significant predictor of outcome in our series (p = 0.007). Children less than 1 of age have a favorable 5-year overall survival (73%) compared with older children (15%). Our results are comparable to other similar studies reporting an overall survival of 70–90% in children less than 1 year of age (Ladenstein et al., 1995; Saito et al., 1997; Bowman et al., 1991; Bernstein et al., 1992). The second important clinical factor is disease stage. In our study, stage IV disease was present in 55% of the children. The 5-year survival was significantly worse for patients with stage IV than for “non-stage IV” patients (13% vs. 47%; p = 0.015), which is consistent with the results of other authors (Saito et al., 1997; Jaffe et al., 1976; Bernstein et al., 1992; Haase et al., 1999).

Stage at diagnosis is closely associated with age. Stage IV disease is present in 68% of children aged 1 year or over, but in only 17% of children under 1 year old. These results are in line with other reports (Saito et al., 1997; Bernstein et al., 1992; Castleberry et al., 1997) describing the presence of stage IV disease in 60 to 70% of children over 1 year old and in 18–25% of children under 1.

Together with stage and age, 1p deletion and NMA are the strongest predictors of unfavorable evolution of the disease. However, NMA and 1p deletion were not routinely investigated over the entire study period, and the percentage of evaluated patients ranged from 43 to 51%.

It has been reported that 1p deletion is the most commonly noted abnormality, affecting up to 80% of neuroblastoma patients (Schleiermacher et al., 1996; Haase et al., 1999). This deletion was detected in 39% of the evaluated patients. Univariate analysis revealed that 1p deletion was a significant predictor of poor outcome in our series (5-year OS 13%; p=0.008). The lower 95% CI (0.67) may attributable to the small number of patients evaluated for 1p deletion. Our results are in line with other similar reports describing 1p deletion as a predictor of poor outcome (Schleiermacher et al., 1996; Haase et al., 1999).
We detected NMA in 26% of the evaluated patients, which is higher than the proportion (15–21%) reported in other studies (Tonini et al., 1997; Hedborg et al., 1992; Lau, 2002). The outcome of N-myc–amplified patients in this series (5-year OS 19%, p=0.014) is worse than that in some studies (OS 26–33%) (Ledenstein et al., 1995; Tonini et al., 1997; Bown et al., 1999) but similar to that in others (Combaret et al., 1998; Tanaka et al., 1998; Lau, 2002) describing an OS of 12–21%.

The difference in NMA proportion and outcome of NMA patients in our report may be attributable to the small number of patients evaluated for NMA.

The difference in the 5-year survival rate for males and females in our series of neuroblastoma patients was not statistically significant (p>0.05)

Univariate analysis in this patient series confirmed a significantly inferior prognosis for NMA, 1p deletion, stage IV, and age of 1 year and over at diagnosis, which is consistent with other similar reports (Evans et al., 1987; Brodeur et al., 1984; Fong et al., 1989; Takeda et al., 1996; Brodeur et al., 1997).

Our report demonstrated that NMA, 1p deletion, stage IV, and age of 1 year and over, were factors pointing strongly to an unfavorable ultimate outcome in NB patients. The aim of future studies of ours will be to assess the prognostic value of all biological and serological parameters in a multivariate analysis, taking into account potential confounding factors such as age and stage.

Acknowledgements — This work was supported by the Ministry of Science, Technology, and Development of Serbia (Grant No. 1541).

REFERENCES


CLINICAL FEATURES, GENETIC FACTORS, AND PROGNOSIS IN NEUROBLASTOMA PATIENTS


PROGNOSTICHE ZNAČAJ KLINICKIH I GENETICKIH FAKTOARA KOD PACIJENATA SA NEUROBLASTOMOM: ISKUSTVO JEDNOG CENTRA

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У Институту за здравствену заштиту мајке и детета Србије, у периоду од јункара 1997 године до јуна 2003 године, дијагностиковано је 47 пацијената оболелих од неуробластома (НБ).

Циљ овог рада био је утврђивање прогностичког значаја клиничких (године пацијената и стадијум неуробластома) и генетичких фактора (делеција 1p и N–мус амплификација) код деце са неуробластомом.

Резултати унивежангијских анализе показали су да је петгодишње преживљавање боље код деце са неуробластомом која су млађа од једне године живота (73% vs. 15%; p=0.007), као и код пацијената са "поп IV" стадијумом тумора у односу на оне са стадијумом IV тумора (47% vs. 13%; p= 0.0015).

Присуство 1p делеције у тумору представља дош прогностички значај за пацијенте (петгодишње преживљавање свега 13% vs. 65% код пацијената без делеције; p=0.008)

Петгодишње преживљавање код пацијената са N–мус амплификацијом је мање него код пацијената без амплификације овог онкогена (19% vs. 58%; p= 0.014).

Анализа клиничких и генетичких фактора у групи од 47 пацијената са неуробластомом показала је да су по редоследу важности најзначајнији прогностички фактори: MYCN амплификација, 1p делеција, стадијум тумора при дијагнози и узраст пацијената.