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PROGNOSTIC VALUE OF CLINICAL, GENETIC AND CYTOGENETIC FINDINGS IN NEUROBLASTOMA PATIENTS FROM SERBIA AND MONTENEGRO

Marija GUĆ-ŠĆEKIĆ[1,3], Marina ĐURIŠIĆ[3], Dragan ĐOKIĆ[3], Dragana VUJIĆ[2,3], Ivan MILOVIĆ[2,3], Slaviša DJURIČIĆ[3], Danijela RADIVOJEVIĆ[3], Tanja LALIĆ[3], and Milena DJURIĆ[2,3]

[1] Faculty of Biology, University of Belgrade,
[2] Faculty of Medicine, University of Belgrade,
[3] Mother and Child Health Institute »Dr Vukan Čupić« , Belgrade, Serbia and Montenegro

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Neuroblastoma (NB) is the most frequent childhood solid tumor. The aim of this study was to report on the prognostic significance of clinical parametres (age and stage), genetic [1p deletion and N-myc amplification (NMA] and cytogenetic results in 47 NB patients diagnosed at the Mother and Child Health Institute of Serbia "Dr Vukan Čupić".Clinical factors evaluated in this studie were age and clinical stage. The 5-year overall survival (OS) was best (73%) in the age group children less than 1 year, compared withthe older children (15%). Stage IV patients had worst outcome (13%) than »non-stage IV« patients (47%).Genetic factors analyzed in this serie of NB patients were: 1p deletion and NMA. 5-year OS was: 65% in the 1p

Corresponding author: Marija Guć-Šćekić,Laboratory of Medical Genetics,Mother and Child Health Institute «Dr Vukan Čupić«,Radoja Dakića 6-8, 11070 Belgrade, Serbia and Montenegro,Tel.: 011 3108 272,e-mail: labgenimd@ptt.yu

deletion negative group and 13% in the 1p36 deletion positive group; 58% in the NMA negative and 19% in NMA positive group.Cytogenetic results showed that normal karyotype, near –diploidy, near-tetraploidy and homogeneously staining regioms (hsr) and double minute chromosomes (dms), together with age over 1 year and stage IV were a very poor prognostic factors.

Key words: neuroblastoma, genetics, cytogenetics

INTRODUCTION

Neuroblastoma (NB) is a childhood solid tumor derived from cells of neural crest.Age at diagnosis and disease stage are considered to be the two most important clinical prognostic factor in NB (COLDMAN et al., 1980; SAITO et al. 1997). Further prognostic factor include: serum markers ferritin, lactate dehydrogenase, and neuron – specific enolase (GRAHAM-POLE et al., 1983; La BROSSE et al., 1980) and recently several genetic features. The most frequent genetic alterations in NB cells are amplification of N-myc oncogene (BRODEUR et al., 1984), deletion of the distal part of the short arm of chromosome 1(p 36) - loss of heterozigosity (LOH) (BRODEUR et al., 1977) and ploidy (LOOK et al., 1991). In most neuroblastoma cells, amplified N- myc is localized in homogeneously staining regions (hsr) of chromosomes and double minute chromosomes (dms), which are cytogenetic manifestation of gene amplification. The aim of this study was to report on the prognostic significance of genetic (1p deletion and N-myc amplification) and cytogenetic results in NB patients diagnosed and treated at the Mother and Child Health Institute of Serbia "Dr Vukan Čupic", taking into account potential confounding factors such as age and stage.

PATIENTS AND METHODS

In the present study, 47 patients with NB were diagnosed at the Mother and Child Health Institute of Serbia "Dr Vukan Čupić" between January 1997 and June 2003.

The disease staging was classified according to the International Neuroblastoma Staging System (INSS) (BRODEUR at al., 1993). The histopatological diagnosis was established on the precise criteria (SHIMADA at al., 1999) after the standard hystopathologic procedure of tumor samples.

Genetic analyses **-** Tumor material was taken either from 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 MYCN amplification were analysed by different techniques: FISH, PCR and additional cytogenetic analysis in some cases.

FISH analyses -A double-target in situ hybridization with centromere D1Z1 (Citocell) and telomere D1Z2 (Citocell) specific probes was performed in order to determine the integrity of 1p.The protocol recommended by Citocell was applied.

N-myc copy number was determined using 2p24/D2Z probe (Q-biogene).

Cytogenetic analyses - Bone marrow cells were cultured for 24 hours and after that treated with colcemid and harvested according to standard procedure. GTG banding was used and the karyotypes were described according to ternational Nomenclature (ISCN, 1995).

Molecular analyses - DNA was isolated from the tumor cells and peripheral blood lymphocytes using standard procedures. LOH 1p was determined 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).

RESULTS

Age at diagnosis and clinical stage -Age at diagnosis was grouped into the following: less then 1 year of age and 1 year of age and more. In the cochort of 47 patients there were 12 (25%) children under the age of 1 year. The 5-year survival rate was 73% in the age group of less than one year and 15% in older children (p=0.007) (Table 1). INSS stage was available for all of 47 patients. Seven patients presented with stage I and II, 13 patients with stage III, 26 patients with stage IV and one patient with stage IVs. Stage I, II, III, and IVs were combined as "non-stage IV" because of the similar survival rates. The 5-year survival rate was 13% for patients with stage IV and 47% for "non-stage "IV" patients (p=0.015) (Table2).

Table 1. Patient Characteristics

Characteristics		All ages		Age groups <1		≥1		p[a]
		No	%	No	%	No	%	
Total		47	100	12	25	35	75	
Sex	Male	33	70	9	75	24	69	0.489
	Female	14	30	3	25	11	31	
Median age (mo)				6		46		
Stage	I&II	7	15	5	42	2	6	14.934
	III	13	28	4	33	9	26	4
	IV	26	55	2	17	24	68	0.005
	IVs	1	2	1	8	0	0	
Histology	NB	41	87	11	92	30	86	0.514
	GN	6	13	1	8	5	14	

p[a] - p value for log rank test; mo - months; NB - neuroblastoma; GN - aganglioneuroblastoma

Genetic Characteristics of Tumors - Chromosome 1p36 and N-myc amplification (NMA) analyses were not routine investigations during the entire study period.

Twenty-three chromosome 1p36.3 investigations using FISH and PCR were performed; 24 were not assessable. Thirteen tumors (56.5%) showed no 1p36.3 aberrations and 10 tumors (43.4%) showed 1p36.3 deletion (Table 2). The 5-year survival rate was 65% in the 1p36 deletion negative group and 13% in the 1p36 deletion positive group (p= 0.008).

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

				5-year				
Prognostic Factor		Total	Death	OS	SE OS	p^a	HR	95% CI
Age group	< 1 Year	12	3	73 %	0.13	**0.007**	1.00	
	≥1 Year	35	29	15 %	0.06		2.27	1.21 - 4.25
Sex	Female	14	8	37 %	0.16	**0.157**	1.25	0.79 - 1.98
	Male	33	24	23 %	0.08		1.00	
Stage								
	Non-stage IV	21	10	47 %	0.12	**0.015**	1.00	
	Stage IV	26	22	13 %	0.07		2.58	1.08 - 6.16
NMA	No	20	9	58 %	0.12	**0.014**	1.00	
	Yes	7	6	19 %	0.17		6.92	1.04 - 46.03
	Non tested	20						
1p deletion								
	No	13	4	65 %	0.14	**0.008**	1.00	
	Yes	10	9	13 %	0.12		4.80	0.67 - 34.63
	Non tested	24						

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

NMA analyses were performed on 27 tumors; 20 were not assessable. N-myc was amplified in 7 (25.9%) tumors.The difference in 5- year survival between the NMA positive and NMA negative group was statistically significant (p = 0.014)(Table2).

Cytogenetic analyses - Cytogenetic analysis was performed on bone marrow cells of 34 patients. In 26 patients with no bone marrow tumor cell infiltration, normal karyotype was found. Of 18 patients with disseminated disease, 17 were over 1 year of age, presented with stadium IV; only one patient in this group was yanger than 1 year, at stadium III of disease (nb 6).In this group the cytogenetic

results were as follows: 8 (44.4%) patients showed normal diploid karyotypes (all at stage IV) and in 10 (29.4%) patients aberrant karyotype was observed (one at stage III and 9 at stage IV). The following aberrant karyotypes were detected: near-diploidy (46±) in mosaic (2 cases- stage IV), near-triploidy (69±) in mosaic (one case-stage IV), near-tetraploidy (92±) in mosaic (4 cases-stage IV), hsr and dms (one case- stage III), del 1p36 in mosaic (one case – stage IV), complex karyotype (one case- stage IV) (Table 3).

DISCUSSION

In this study we investigated the prognostic significance of genetic (1p deletion and NMA), cytogenetic and clinical factors such as stage and age in 47 patients with NB.

Patients were devided into two age groups: children under 1 year of age and older children over 1 year of age. The 5-year overall survival was best (73%) in the age group of less than 1 year, compared to the older children (15%).Our results are in line with other studies reporting the overall survival of 70-90% in children les then 1 year (BERNSTEIN et al., 1992; SAITO et al., 1997).

The second clinical factor evaluated in this studie was clinical stage. Stage IV patients had worst outcome (13%) than "non-stage IV" patients (47%) , which is consistent with other reports (BeRNSTEIN et al. 1992; HAASE et al. 1999).

1 p 36.3 deletion is the most frequent abnormality in NB patient (SCHLEIERMACHER et al., 1996). In our group of 23 evaluated patients, 1p deletion was present in 39%.

Univariate analysis showed that 1p deletion was a significant predictor of poor outcome in our serie what is in line with other similar reports (CARON et al. 1996; CHEIRMACHER et al. 1996).

The N-myc oncogene is located on chromosome 2p and is amplified in greater than 40%of disseminated neuroblastomas (BRODEUR et al., 1984; MATTHAY et al., 1997). In our serie of 27 patients, NMA was detected in 26% of the patients.The 5-year survival in NMA positive patients was worst (19%) than in NMA negative patients (58%). Our results are comparable with the results of other reports (TANAKA et al., 1998;LAU 2002).

Univariate analysis in this serie of patients showed that NMA, 1p deletion, stage IV and age 1 year and over were a significant predictor of poor prognosis in NB patients.Cytogenetic analysies performed on bone marrow cells of 18 NB patients with disseminated tumors (17 with stage IV and one with stage III) showed that normal karyotype, near diploidy, near tetraploidy and hsr and dms together with age over 1 year and stage IV were very poor prognostic factors (with exeption of patient nb 9 who had very good response to chemotherapy).

In one patient (nb 8) with stage IV we found triploidy in mosaic in bone marrow cells. This patient is still alive with good response to chemotherapy. Diploidy or tetraploidy have already been identified in advanced stage of NB (LOOK et al. 1991).

Normal karyotype with absence of 1p deletion and MYCN amplification in patients younger then 1 year, presented with early stage of NB and no infiltration, were associated with relatively good prognosis.

More patients need to be evaluated for cytogenetic analysies in order to confirm our first cytogenetic results presented here.

The present study confirms the importance of combined application of cytogenetic and molecular techniques in accurate patient assigment to risk groups, so that treatment strategies can be more effectively undertaken.

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UTICAJ KLINIČKIH, GENETIČKIH I CITOGENETIČKIH PARAMETARA NA ISHOD BOLESTI KOD PACIJENATA SA NEUROBLASTOMOM IZ SRBIJE I CRNE GORE

Marija GUĆ-ŠĆEKIĆ[1,3], Marina ĐURIŠIĆ[3], Dragan ĐOKIĆ[3], Dragana VUJIĆ[2,3], Ivan MILOVIĆ[2,3], Slaviša DJURIČIĆ[3], Danijela RADIVOJEVIĆ[3], Tanja LALIĆ[3], i Milena DJURIĆ[2,3]

[1] Biološki fakultet, Univerziteta u Beogradu,
[2] medicinski fakultet, Univerziteta u Beogradu,
[3] Institu za majku i dete »Dr Vukan Čupić« , Beograd, Srbija i Crna Gora

I z v o d

Cilj ovog rada bio je ispitivanje prognostičkog značaja kliničkih (stadijum bolesti i uzrast), genetičkih [1p delecija i N-myc amplifikacija (NMA)] i citogenetičkih faktora u uzorku bolesnika sa neuroblastomom (NB) iz Srbije i Crne Gore. Rezultati su pokazali da klinički faktori kao što su: uzrast pacijenta preko 1godine i IV stadijum bolesti, zatim prisustvo 1p36 delecije i NMA, zajedno sa normalnim kariotipom, “near”- diploidijom ili “near”- tetraploidijom, predstavljaju faktore visokog rizika za preživljavanje NB pacijenata.

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