ORIGINAL ARTICLE

Prognostic value of the International Neuroblastoma Pathology Classification in Neuroblastoma (Schwannian stroma-poor) and comparison with other prognostic factors: a study of 182 cases from the Spanish Neuroblastoma Registry

Octavio Burgues · Samuel Navarro · Rosa Noguera ·
Antonio Pellín · Amparo Ruiz · Victoria Castel ·
Antonio Llombart-Bosch

Abstract In addition to clinical and biological factors, further valuable prognostic information in neuroblastoma (Schwannian stroma-poor) (NB) patients is provided by the histopathologic analysis and the application of the International Neuroblastoma Pathology Classification (INPC) system. The objective of this study was to assess the prognostic impact of the INPC classification in a series of NB (Schwannian stroma-poor) and its relation with other prognostic factors. One hundred eighty-two cases of NB were collected from the files of the Spanish Neuroblastoma Registry. Slides were reviewed, and NB cases were grouped into favorable and unfavorable categories according to INPC criteria, taking into account morphological features (mitosis–karyorrhexis index, histological subtype) and patient's age at diagnosis. Other pathological [presence of calcifications, tissular components, and number of mitotic cells per 10 high-power field (HPF)], immunohistochemical (P-glycoprotein and Ki-67 protein expression) and genetic (MYCN amplification and chromosome 1p deletion) features were also studied. Statistical analyses of overall survival with Kaplan–Meier curves and a multivariate study using Cox regression were performed (40.3% of NBs were considered favorable and 59.7% unfavorable).

Unfavorable NB showed a mean survival time of 57 months compared with 89 months in favorable cases. Advanced stage, more than ten mitoses per 10 HPF, Ki-67 expression in more than 30% of tumor cells, MYCN oncogene amplification and chromosome 1p deletion were observed more frequently in unfavorable NB. The Cox regression analysis demonstrated that clinical stage (International Neuroblastoma Staging System stage 4) and histological subtype (undifferentiated NB) were the most important factors that influence the overall survival ($p<0.001$). INPC classification results are major prognostic indicators in NB and should be considered in the therapeutic stratification of NB patients.

Keywords Neuroblastoma · Neuroblastic tumors · Pediatric tumors

O. Burgues · S. Navarro (✉) · R. Noguera · A. Pellín · A. Ruiz ·
A. Llombart-Bosch
Departamento de Patología, Facultad de Medicina de Valencia,
Avda. Blasco Ibañez, 17,
E-46010 Valencia, Spain
e-mail: samuel.navarro@uv.es

V. Castel
Pediatric Oncology Unit, Hospital Universitario "La Fe",
Valencia, Spain

Introduction

Classically, neuroblastoma (NB) (Schwannian stroma-poor) prognosis is based on two clinical parameters: patient's age at diagnosis and tumor stage [7]. Several established molecular prognostic parameters, such as DNA content (ploidy) [39], MYCN oncogene amplification [45], allelic loss in the short arm of chromosome 1 (1p) [15], and gain of genetic material in chromosome 17 [4, 10], have been progressively introduced as further prognostic indicators. Telomerase activity is being analyzed as a strong new prognostic molecular parameter in NB [42, 43].

Valuable prognostic information is also provided by the histopathologic analyses. Shimada et al. [49] proposed a

classification system for neuroblastic tumors based on the proportion of Schwannian stroma, with a prognostic categorization in favorable or unfavorable subgroups, combining morphological features with the patient's age at diagnosis. Subsequently, Joshi et al. [31, 32] proposed a different age-linked prognostic categorization of NB into two risk groups supported by a new histological grading system, which was later modified [34]. Finally, the system of Shimada et al. [49] was adapted by the International Neuroblastoma Pathology Committee to elaborate on the International Neuroblastoma Pathology Classification (INPC) [47, 48], which standardized a terminology and morphological criteria to classify neuroblastic tumors with prognostic significance.

The immunohistochemical procedures allow the consideration of additional features with actual prognostic value in NB such as P-glycoprotein (the product of the multidrug resistance gene, MDR-1) [13] and the overexpression of the neural growth factor receptor-associated protein Trk-A [5]. The determination of cell proliferation markers such as Ki-67 protein also provides prognostic information on these tumors [44].

In this paper, we review the morphological features of 182 NBs (Schwannian stroma-poor) collected from the files of the Spanish Neuroblastoma Registry, applying the INPC criteria, with the aim of assessing the prognostic impact of this classification and its relation with other clinical, morphological, and biological prognostic factors.

Materials and methods

In this study, 209 NBs (Schwannian stroma-poor) with pathologically valuable material were retrieved from the files of the Spanish Neuroblastoma Registry. The cases were collected between January 1992 and December 1999 from the 26 hospitals enrolled in the Spanish Cooperative Neuroblastoma Study N-II-92 [12]. Each case was diagnosed and staged in accordance with the International Neuroblastoma Staging System (INSS) [6, 7]. Samples of fresh and frozen tumor tissue from the primary tumor or a metastatic site (if it was not available for the primary tumor) were submitted to this reference laboratory. Tumor specimens taken after chemotherapy were excluded from the study. Clinical data from each patient were collected, including sex, date of birth, age at diagnosis, tumor location, INSS stage, clinical follow-up, and final outcome (data available for 182 patients). The cutoff date for the study was June 2000.

Histopathologic analysis Standard procedures were used to prepare hematoxylin-eosin (H&E) sections after paraffin embedding of tumor tissue fixed in 10% buffered formaldehyde. All slides in each tumor were examined by two of the authors with no knowledge of either clinical or genetic abnormalities, and the histological diagnosis based on INPC recommendations [47] was established. The mitosis–karyorrhexis index (MKI) was determined in each tumor according to INPC criteria. Patient's age was taken into account to classify each case into a prognostic subset, favorable or unfavorable, following INPC prognostic categorization [48] (Table 1). Other morphological features considered were the diverse parameter of tissular components found in the slides: percentage of viable tumor cells, percentage of Schwannian stroma, percentage of necrotic tissue (including hemorrhagic and calcified areas) and percentage of other elements (including inflammatory cells, normal tissue, and non-Schwannian stroma), the presence of tumor calcifications, and the number of mitotic cells per 10 HPF.

Immunohistochemical expression of Ki-67 protein (MIB-1; Immunotech; 1:50) was analyzed in all cases following the streptavidin–biotin peroxidase method (LSAB, DAKO) [22]. Antigen retrieval was obtained with microwave heating in a solution of citrate buffer (pH 6). The same technique was performed to assess P-glycoprotein expression using the monoclonal antibody JSB-1 (Centocor; 1:50).

Genetic studies MYCN oncogene amplification was determined in all cases by means of Southern blot analysis of total DNA extracted from frozen tumor samples and fluorescence in situ hybridization (FISH) analysis with a DNA probe [N-myc (2p24) red/Alphasatellite 2 Dual-Color, Q-Biogene/mpbio]. To study chromosome 1 status, FISH analysis was performed on tumor touch imprints in 72 NBs

Table 1 Prognostic evaluation of neuroblastoma according to the INPC [48]

INPC type	Age (years)	INPC subtype	MKI	Prognostic group
NB	<1.5	Undifferentiated	Any MKI	Unfavorable
		Poorly differentiated/differentiating	Low/Medium	Favorable
		Any subtype	High	Unfavorable
	1.5–5	Undifferentiated/Poorly differentiated	Any MKI	Unfavorable
		Differentiating	Low	Favorable
		Any subtype	Medium/High	Unfavorable
	>5	Any subtype	Any MKI	Unfavorable

using a chromosome 1 centromeric (1p36 Midi Satellite Probe; green, Q-Biogene/mpbio) and subtelomeric [Chr.1 Classical Satellite (1qh), red, Q-Biogene/mpbio] probe by means of standardized methods [2]. The number of signals was counted in 100 tumor cell nuclei.

Statistical analysis All clinical, morphological, and biological data obtained for each case were filed in a computerized database. A descriptive analysis was performed for each variable included, and associations among these variables were analyzed using the appropriate statistical method (χ^2, Mann–Whitney test, and Kruskal–Wallis test). The Kaplan–Meier method was used to estimate survival rates, and multivariate study using Cox regression procedure was performed. p values ≤0.05 were regarded as statistically significant.

Results

Analysis of clinical, biological, and morphological features and their influence on patient's outcome and survival

The clinical, morphological, and biological features of tumors with known follow-up data are listed in Table 2, which also includes the results for their final outcome, p value for bivariate analysis, and statistical test used. Information on the clinical course of 182 patients was available: 135 (74.2%) were alive at the last follow-up, whereas 47 (25.8%) had died of the disease or as a complication of the treatment. Mean observation time was 33.54 months. The 3-year estimated survival rate was 72.9%, with a mean survival rate of 73 months.

The mean age at the moment of diagnosis was 20.5 months. Patients younger than 18 months presented a better outcome and a more prolonged survival (87 months) than older ones. The intermediate age group (1.5–5 years) had the worst survival (44 months), and 51.9% of the patients were dead. Mean age at diagnosis in patients who remained alive was 17 months, nearly half of the patients who died (31.3 months).

Tumors presented in all stages. The number of deaths increased significantly with higher stages. With a mean survival rate of 35 months, 59.3% of INSS stage 4 patients were dead. Behavior of INSS stage 4s NBs was similar to INSS stage 1 tumors (Fig. 1).

MYCN amplification was observed in 29 NBs (16.7%) and was significantly associated with a worse outcome (58.6% were dead) and shorter survival (40 months) than patients without amplification of the gene (17.2% were dead; mean survival, 79 months). By FISH analysis, 27.3% of the NBs studied showed loss of genetic material in the short arm of chromosome 1, a feature that was also related with patient outcome and survival: 44.4% of the patients with this alteration died, with a mean survival rate of

32 months, in contrast to patients without 1p deletion, who for the most part remained alive (87.5%) (mean survival rate, 74 months). All 12 tumors with MYCN amplification in which chromosome 1 was analyzed showed loss of genetic material. INSS stage data were available in 11 of them: 8 (72.7%) were stage 4 tumors, and 3 (27.3%) were stage 3. All but one (91.7%) showed a high MKI. Clinical follow-up information was available in nine of them, and seven (77.8%) were dead.

The immunohistochemical expression of P-glycoprotein was found in 10.3% of 156 NBs analyzed. The percentage of dead patients in the group with P-glycoprotein expression (31.3%) was higher than in the negative group (25.7%), but differences were not statistically significant.

Morphological features According to the INPC diagnostic criteria, 111 (61%) tumors were diagnosed as poorly differentiated NB (PD-NB), 35 (19.2%) as undifferentiated NB (U-NB), and 12 (6.6%) as differentiating NB (D-NB). The remaining 24 (13.2%) cases were tumors with a definitive generic diagnosis of NB, but in which the subtype was impossible to determine because of the paucity of tumor cells or the degree of artifact. These last cases fit into the not otherwise specified (NOS-NB) category. A sample of each subtype is showed on (Fig. 2).

Nearly two-thirds (62.9%) of U-NBs with clinical follow-up had a fatal outcome, with a mean survival rate of 33 months, significantly shorter than in the other subtypes (58–81 months). The best outcome was for D-NB, with only one (8.3%) dead patient. Behavior of tumors regarded as "NOS" was similar to PD-NB, with less than 20% of dead patients (18% in PD-NB, 16.7% in NOS-NB) and a mean survival rate of 80 months (Fig. 3).

Necrosis was significantly related to a higher proportion of dead patients (15.4% in tumors with necrosis vs 8% in tumors without this feature). Inversely, the presence of Schwann cells was associated with a better outcome. A high MKI, more than 6 mitoses per 10 HPF, or Ki-67 expression in more than 30% of tumor cells were features that determined a significant rise in the proportion of dead patients, with a concomitant drop in the mean survival time. We found no differences in outcome between patients whose tumors showed a low or intermediate MKI, most of them (87.9% with low MKI, 85.3% with intermediate MKI) remaining alive at last follow-up.

Multivariate analysis Tumor stage and histological subtype of NB were the factors having a highly significant influence on survival rate. Moreover, INSS stage 4 and undifferentiated subtype of NB were the features with a more negative effect over hazard rate compared with the remaining stages and histological subtypes. Results of the Cox regression procedure are resumed in Table 3.

Table 2 Clinical, morphological, and biological data for each tumor related to outcome

Characteristics	n	%	Alive		Dead		p^a	Statistic
			n	%	n	%		
Sex								
Boys	104	57.1	76	73.1	28	26.9	0.696	χ^2
Girls	78	42.9	59	75.6	19	24.4		
Age (years)								
<1.5	115	63.2	100	87	15	13	**<0.001**	χ^2
1.5–5	52	28.6	25	48.1	27	51.9		
>5	15	8.2	10	66.7	5	33.3		
Tumor location								
Adrenal	98	55.1	77	78.6	21	21.4	Not performed	Not performed
Paravertebral (abdominal)	28	15.7	23	82.1	5	17.9		
Mediastinal	23	12.9	18	78.3	5	21.7		
Pelvic	7	3.9	7	100	0	0		
Cervical	3	1.7	2	66.7	1	33.3		
Disseminated (no primary site specified)	19	10.7	4	21.1	15	18.9		
Stage (INSS)								
1	47	25.8	46	97.9	1	2.1	**<0.001**	Kruskall–Wallis
2	17	9.3	13	76.4	4	23.6		
3	42	23.1	33	78.6	9	21.4		
4	54	29.7	22	40.7	32	59.3		
4s	22	12.1	21	95.5	1	4.5		
Histologic subtype (INPC diagnosis)								
Undifferentiated NB	35	19.2	13	37.1	22	62.9	**<0.001**	χ^2
Poorly differentiated NB	111	61	91	82	20	18		
Differentiating NB	12	6.6	11	91.7	1	8.3		
NB, NOS	24	13.2	20	83.3	4	16.7		
Tumor calcifications								
Yes	51	28	40	78.4	11	21.6	0.413	χ^2
No	131	72	95	72.5	36	27.5		
Mitotic count (per 10 HPF)								
0	1	0.6	1	100	0	0	**<0.001**	Mann–Whitney
1–5	58	37.4	51	87.9	7	12.1		
6–10	46	29.7	32	69.6	14	30.4		
>10	50	32.3	29	58	21	42		
MKI								
Low	58	36.9	51	87.9	7	12.1	**<0.001**	Mann–Whitney
Intermediate	34	21.7	29	85.3	5	14.7		
High	65	41.4	34	52.3	31	47.7		
Ki-67 protein expression (%)								
≤10	41	23.6	35	85.4	6	14.6	**<0.001**	Mann–Whitney
11–30	70	40.2	58	82.9	12	17.1		
31–50	31	17.8	17	54.8	14	45.2		
>50	32	18.4	18	56.3	14	43.8		
MYCN status								
NO amplificated	145	83.3	120	82.8	25	17.2	**<0.001**	Mann–Whitney
Amplificated	29	16.7	12	41.1	17	58.6		
Del 1p								
Yes	18	27.3	10	55.6	8	44.4	**0.005**	χ^2
No	48	72.7	42	87.5	6	12.5		
p-glicoprotein expression								
Yes	16	10.3	11	68.8	5	31.3	0.634	χ^2
No	140	89.7	104	74.3	36	25.7		
Prognostic categorization (INPC)								
Favorable	64	39.5	60	93.8	4	6.2	**<0.001**	Mann–Whitney
Unfavorable	98	60.5	58	59.2	40	40.8		

[a]Significant associations (p value ≤0.005) in bold

STAGE

□ 4s

■ 4

■ 3

■ 2

■ 1

Cumulative survival
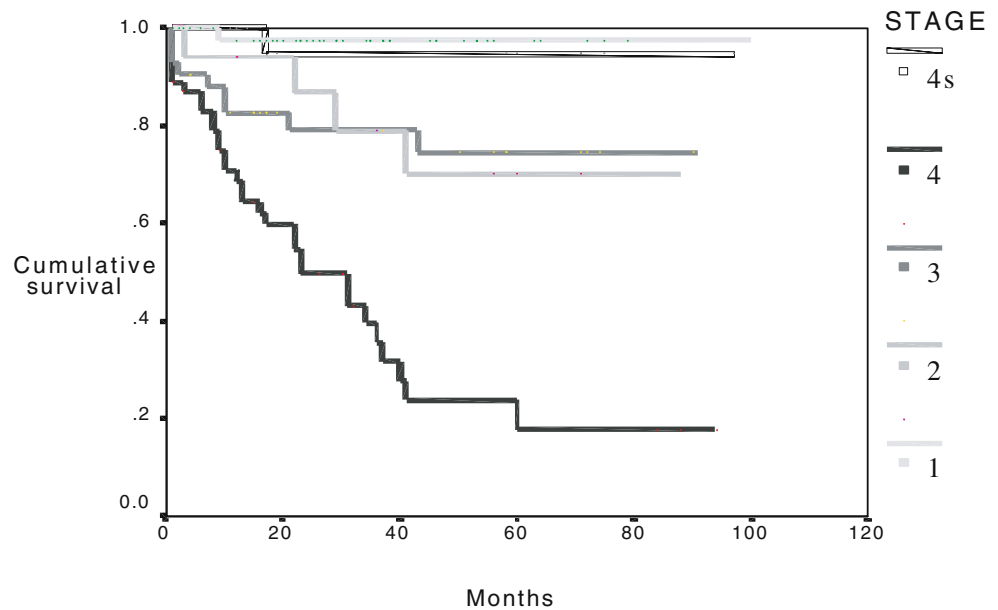
Months

Relations between histological subtype of NB and other clinical, morphological, and biological parameters

Poorly differentiated NB and NOS-NB were diagnosed frequently in the first 18 months of life (70%). In contrast, 60% of D-NBs and 63.2% of U-NBs were diagnosed in children older than 1.5 years.

Nearly two thirds of the U-NBs (63.2%) presented as disseminated (INSS stage 4) tumors, whereas PD-NBs and

D-NBs were usually localized (INSS stages 1 and 2). Most INSS stage 4s tumors were PD-NBs (66.7%).

The presence of Schwann cells was only significant in D-NB, reaching a mean proportion of 10.3% in this subtype. On the contrary, necrosis was rare in these tumors (2.3%), but reached a mean proportion of 18.2% in slides from U-NB. Tumor calcifications were related to the degree of differentiation of NB, being detected in 60% of D-NBs but in only 18.4% of U-NBs. The proliferative activity

Fig. 2 Histological subtypes of neuroblastoma (Schwannian stroma-poor): **a** undifferentiated NB with high MKI; **b** poorly differentiated NB with Homer–Wright rossettes; **c** differentiating NB; **d** NOS-NB with important artifact. All images are H & E, ×40
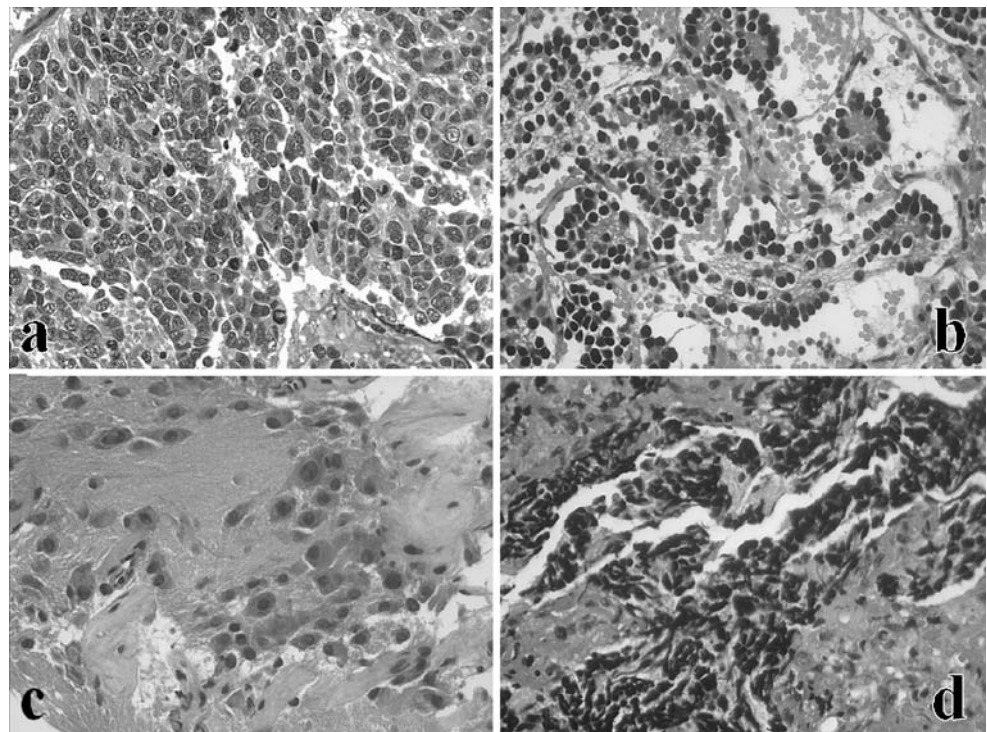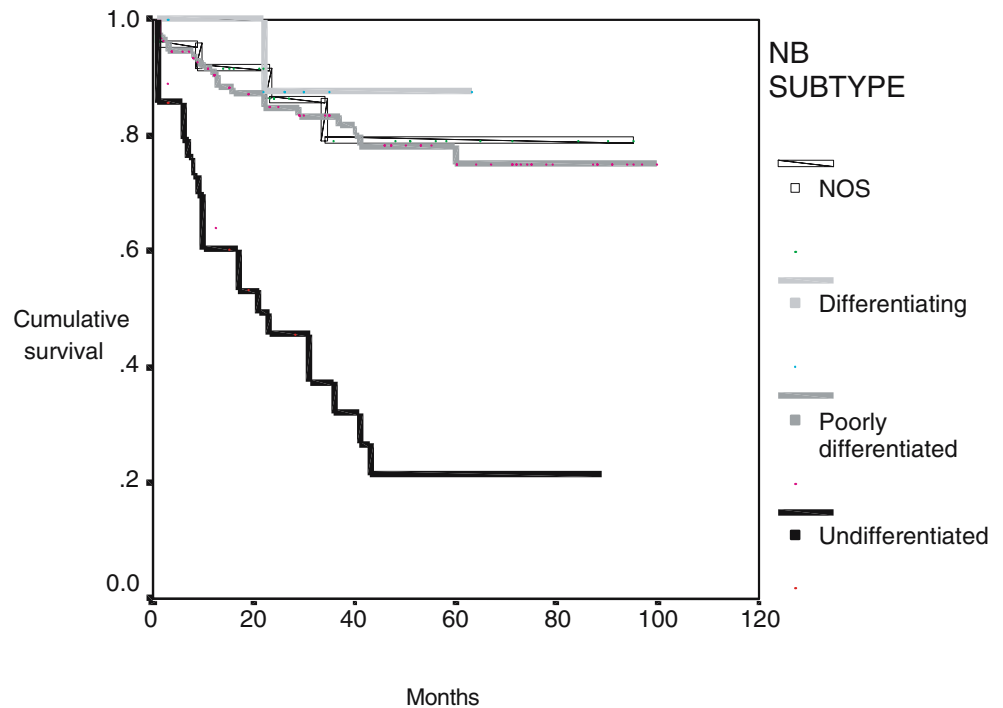
Fig. 3 Variations in survival with regard to the histological subtype of NB

offered marked differences among the NB subtypes, being significantly higher in U-NB compared with the remaining categories. With detail, 72.7% of U-NBs showed a high MKI, more than 10 mitoses were found in 62.5% of them, and Ki-67 nuclear labeling reached a mean percentage of 47.6%. In contrast, D-NBs showed a low MKI (86.7%), less than 5 mitoses (93.3%), and a mean percentage of Ki-67 nuclear labeling of 11.8%.

MYCN oncogene amplification and 1p deletion were found significantly more frequently among U-NB than in remaining subtypes: 45.9% of these tumors showed MYCN amplification, but this feature was found in 16% of NOS-NBs, 9% of PD-NBs, and none in D-NB. 1p deletion was present in seven (70%) of 10 U-NBs, but in only 20–22% in the remaining categories. We could not find any significant differences on P-glycoprotein expression with regard to the histological subtype of NB.

Analysis of the prognostic INPC categorization

Following the INPC criteria, we established the favorable or unfavorable category in 162 patients with available follow-up data. Of the 162 patients, 39.5% of them fitted into the favorable group, whereas the remaining 60.5% were considered as unfavorable. We found an evident association between the prognostic group and outcome: most patients (93.8%) in the favorable group remained alive at last follow-up (Fig. 4), and their mean survival rate (89 months) was significantly higher than those in the unfavorable group (57 months) (Fig. 5). The INPC prognostic system offered a high sensitivity (90.9%), with most of the dead patients located in the unfavorable group (40 of 44 dead patients). However, specificity of this prognostic scheme was low (50.8%) because in this series, half of the patients regarded as unfavorable remained alive at last follow-up.

Table 3 Cox regresion model

Variable ▼	β ▼	p ▼	Exp (β) ▼
Stage	Reference category = 1; p value <0.001		
2	0.951	0.502	2.588
3	1.090	0.337	2.975
4	2.919	0.005	18.539
4s	0.803	0.571	2.233
Histological subtype of NB	Reference category: undifferentiated NB p value <0.001		
PD-NB + D-NB	−1.266	0.003	0.281
NOS	−0.675	0.517	0.508
Cox regresion equation	$H(t) = H_0(t)\, 2.588^{(Stage=2)}\, 2.975^{(Stage=3)}\, 18.539^{(Stage=4)}\, 2.233^{(Stage=4s)}$ $0.281^{(Subtype\ =PD\text{-}NB\ or\ D\text{-}NB)}\, 0.508^{(Subtype=NOS)}$		
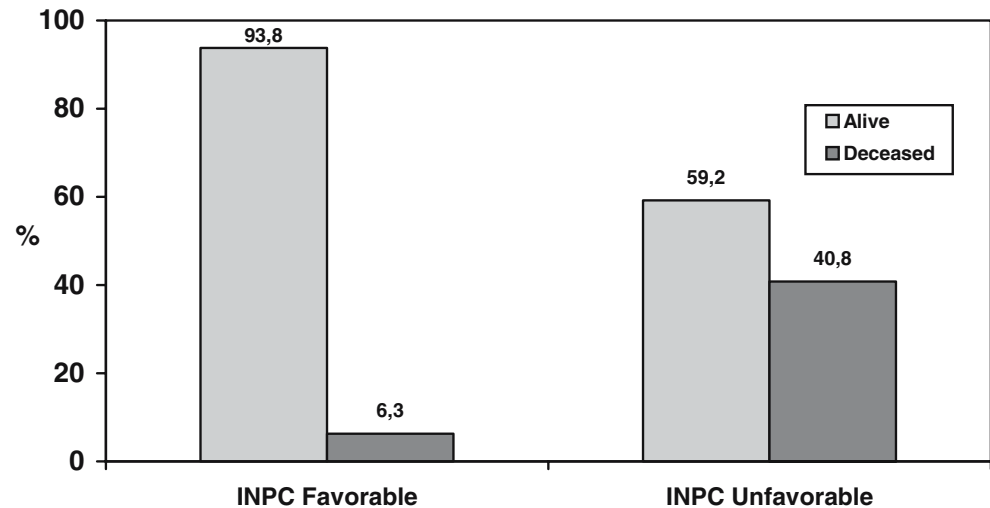
The tumor stage was significantly related to INPC progno-sis. Progression in stage implied a higher proportion of unfavorable cases, which reached 89.3% in INSS stage 4 tumors. Results for INSS stage 4s tumors were similar to those of INSS stage 1 tumors, with two thirds of the cases categorized as favorable (65.2% in stage 1; 68.4% in stage 4s).

Regarding the histological composition of the samples, only the proportion of Schwann cells was significantly higher among favorable tumors. The proportion of favor-able cases was higher among tumors in which calcifications were found (48.3% vs 36.5% of INPC favorable tumors in the group without calcifications), but differences were not significant.

Tumors with less than five mitoses per 10 HPF were mainly classified as favorable (70.9%), whereas nearly all tumors (98.2%) with more than ten mitoses per 10 HPF belonged to the unfavorable group. The Ki-67 nuclear expression reached a mean value of 40% in unfavorable cases, significantly higher than the mean expression found in favorable tumors (15.8%). In fact, most cases (96.5%) with Ki-67 nuclear expression in more than 30% of tumor cells fell into the unfavorable group.

The presence of MYCN gene amplification or 1p deletion was significantly related to INPC prognosis. Nearly all tumors with MYCN gene amplification were in the unfavorable group (93.1%), the same as for NB showing 1p deletion (84.2% of tumors classified as unfavorable).

Discussion

It has been assumed that NB follows a maturative sequence characterized by a progressive differentiation of tumor cells together with the development of stroma composed mainly of Schwann cells [30, 46]. First attempts to establish a classification of these tumors considered cell differentiation
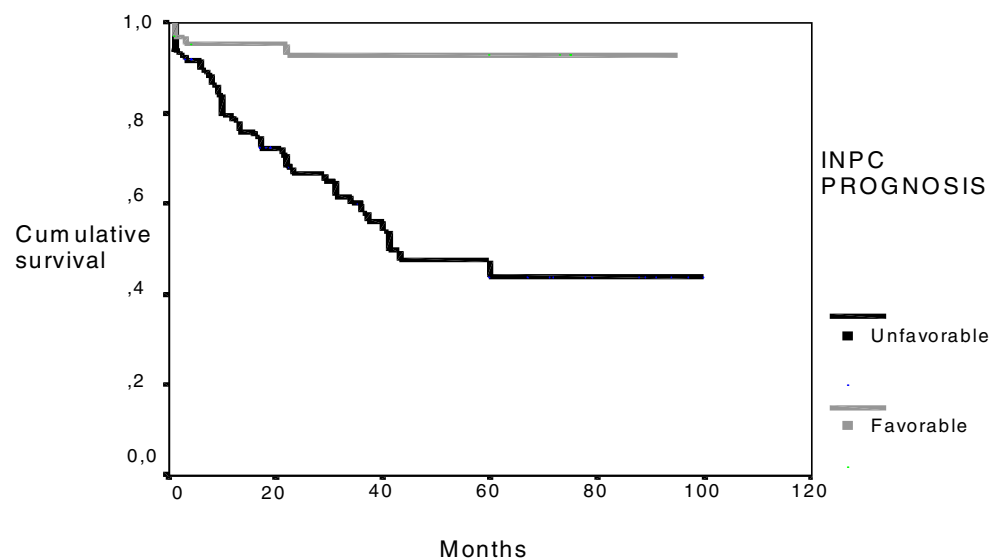
as the main morphological parameter with prognostic value [3, 28, 40]. Tumor cell differentiation and development of Schwannian stroma were the basis for the classification system and age-linked prognostic categorization proposed by Shimada et al. [49], whose prognostic value was confirmed in subsequent reports [14, 33]. This model was adapted and modified by the International Neuroblastoma Pathology Committee to establish a standard terminology and morphological criteria for the diagnosis of NB, offering a reproducible classification system (the INPC system) that has prognostic value and biological relevance [47, 48].

In this article, we have reviewed 182 NBs (Schwannian stroma-poor) collected from the files of the Spanish Neuroblastoma Registry focusing on pathological features. The INPC diagnostic and prognostic system was applied with the aim of assessing the true prognostic value of this system in our series and its relation with other prognostic factors.

We found patient outcome to be related with histological subtype of NB in such a way that U-NB had a death rate significantly higher than in the other subtypes, and their mean survival time was cut in half. Using a multivariate analysis (Cox regression), the histological subtype of NB was the second most influential factor on survival, closely following tumor stage. INSS stage 4 and undifferentiated histology were the features with a more negative effect on hazard rate.

Tumor necrosis has been related to a worse prognosis in NB [14]. In this series, the proportion of dead patients was significantly higher among tumors with necrosis, which mainly fit into the undifferentiated or "NOS" category. In contrast, the presence of Schwann cells has been related with a better outcome in NB [47, 48]. We found a better outcome for patients whose tumors contained Schwann cells, but this cell type was practically restricted to D-NB. Tumor calcifications have an uncertain biologic significance in NB, but they have been related to a better prognosis in some reports [14, 32], including the INPC report [47]. In our series, they appeared significantly more frequently in D-NB, but differences in outcome with regard to their presence were not significant.

Joshi et al. [32] proposed a grading system for NB based on mitotic rate and the presence of calcifications, these being the morphological factors found to have more impact on prognosis. In our series, mitotic count was significantly related to patient's outcome in such a way that more than six mitoses per 10 HPF implied a remarkable rise in death rate, which was 42% in tumors with more than ten mitoses per 10 HPF. We found mitotic rate to be also related to the histological subtype of NB, being especially high in U-NB. Assessment of mitotic rate in NB can be difficult in practice because of the resemblance of mitotic and karyorrhectic cells. For this reason, the MKI was proposed. These data are obtained from the sum of mitotic and karyorrhectic figures out of 5,000 tumor cells [49]. MKI was one of the

features that determined the prognosis in the original Shimada system and, after subsequent reports confirmed its prognostic value [31], it has been maintained as a prognostic factor in the INPC system [48]. In our series, high MKI tumors had a death rate close to 50%, whereas most patients with low or medium MKI tumors remained alive at last follow-up and had a significantly higher mean survival rate. Almost all D-NBs showed a low MKI, whereas this index was high in most U-NBs. Ki-67 protein is an excellent marker for assessing the proliferative activity in tissue [9]. We found significant differences in patient outcome and survival with regard to mean nuclear Ki-67 expression, to the effect that death rate increased markedly when expression was 30% or higher, with a concomitant decrease in mean survival time. In previous reports, low Ki-67 protein expression in NB was related to a better prognosis [24, 44]. U-NB showed a high Ki-67 protein expression (mean value near 50%), significantly higher than in PD-NB and in D-NB (lower than 30%).

Recently, some reports have described prominent nucleoli to be a sign of unfavorable tumor biology and MYCN amplification in U-NB and PD-NB [1, 37]. We are reviewing the slides collected in the Spanish Neuroblastoma Registry to evaluate this parameter (manuscript in preparation).

It is well established that NB of any stage diagnosed in infants have a better prognosis than those diagnosed in children at the same stage [17, 19, 25, 29]. In fact, the main NB prognostic categorization schemes based on morphological parameters were age-linked systems [32, 48, 49].

Tumor stage is also an independent prognostic factor in NB: localized tumors have longer survival rates than disseminated ones [17, 19]. In the multivariate analysis, we found that INSS stage 4 was the parameter with a more negative effect on hazard rate. U-NB appeared as disseminated tumors in the majority of patients, similar to those categorized as NOS, whereas localized tumors predominated among PD-NB and D-NB. INSS stage 4s tumors represented 10% of all NB in our series, a similar proportion to that previously reported [18, 29], and for the most part, they fit into the PD-NB category.

MYCN gene amplification correlates with poor prognosis in NB [8, 16, 20, 45, 52]. We confirmed this fact in our series, in that almost 60% of the patients with MYCN-amplified tumors were deceased, and moreover, we also found a clear relation between this alteration and the histological subtype of NB. MYCN gene amplification appeared in half of the U-NB cases. Previous reports described the presence of a typical phenotype in these amplified NB, being for the most part undifferentiated stroma-poor tumors with a high proliferative activity [21, 38, 50, 52].

Only 72 tumors in our series were studied by means of FISH analysis to assess chromosome 1p status, deletion

being found in 28% of them. As has been previously reported [11, 15, 20, 27, 35], del 1p was related to a worse outcome and a shorter survival rate. We detected this alteration in 70% of the U-NB.

We have classified NB into favorable or unfavorable groups according to the INPC prognostic system. INPC prognosis was significantly related to patient's outcome in such a way that most patients in the favorable group remained alive at last follow-up, whereas the proportion of dead patients was high in the unfavorable group. Mean survival rate for favorable patients was significantly higher than that of unfavorable patients. The INPC prognostic system had a high sensitivity in this series (90.9%): most of the dead patients were in the unfavorable group. However, the specificity of this system was low (50.8%) because half of the patients classified as unfavorable remained alive at last follow-up. The prognostic value of the INPC system has been confirmed in several reports [1, 23, 38, 51]. Recently, the Localised Neuroblastoma European Study Group (LNESG) have shown that the INPC prognostic categorization has a significant impact on outcome prediction in INSS stage 2 localized peripheral NB [41].

In addition to factors that determine INPC prognosis (histological type, MKI, and patient's age at diagnosis), we have found significant associations between the prognostic group and other clinical, morphological, and biological parameters. Progress in the tumor stage is associated with a rise in the proportion of unfavorable cases. Results for INSS stage 4s tumors were similar to those of stage 1 NB and, as is described in the literature, most of them were tumors with favorable features [26, 36]. The rise in mitotic count or in Ki-67 protein labeling index also implied a higher proportion of unfavorable cases. We found MYCN gene amplification and 1p deletion to be significantly more frequent in the unfavorable group. Nearly all tumors with one of these alterations were located in the unfavorable group.

Based upon the present analysis, we can state that, although molecular biology studies have an increasing significance, the histopathologic study is still an important procedure in the study of NB and provides valuable prognostic information. We have confirmed the high sensitivity of the INPC prognostic system, observing that NB in the unfavorable group displays a worse outcome. We have also corroborated the association of clinical, morphological, and biological parameters with their clinical outcome. In this series, NB located in the unfavorable group presented features related to a worse prognosis, INSS stage 4 and undifferentiated histology being the features with a more negative impact on survival.

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