

The International Neuroblastoma Pathology Classification (the Shimada System)

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BACKGROUND. The International Neuroblastoma Pathology Committee, which is comprised of six member pathologists, was convened with the objective of proposing a prognostically significant and biologically relevant classification based on morphologic features of neuroblastic tumors (NTs) (i.e., neuroblastoma, ganglioneuroblastoma, and ganglioneuroma).

METHODS. A total of 227 cases were reviewed. Consensus diagnoses from morphologic features (criteria described separately) based on five of six or six of six agreements by the reviewer pathologists were used for prognostic analysis. Prognostic effects of morphology, both individual and in combination, taken in conjunction with age (Shimada classification, histologic grade, and risk group), were analyzed.

RESULTS. Approximately 99% of cases (224 of 227) had consensus diagnoses for categorization: neuroblastoma (Schwannian stroma-poor), 190 cases; ganglioneuroblastoma, intermixed (Schwannian stroma-rich), 5 cases; ganglioneuroma (Schwannian stroma-dominant) maturing, 1 case; ganglioneuroblastoma, nodular (composite Schwannian stroma-rich/stroma-dominant and stroma-poor), 19 cases; and NT-unclassifiable, 9 cases. For the NTs, subtype (93% consensus: undifferentiated, 6 cases; poorly differentiated, 155 cases; and differentiated, 15 cases), mitosis-karyorrhexis index (90% consensus: low, 94 cases; intermediate, 40 cases; and high, 37 cases), mitotic rate (75% consensus: low, 89 cases; high, 50 cases; and not determined, 4 cases), and calcification (100% consensus: yes, 110 cases and no, 80 cases) were recorded. Statistical analysis demonstrated that the Shimada classification system (90% consensus; 3-year event free survival: 85% for the group with favorable histology and 41% for the group with unfavorable histology; $P = 0.31 \times 10^{-9}$) had a significantly stronger prognostic effect than individual features and other combinations.

CONCLUSIONS. The International Neuroblastoma Pathology Classification, a system based on a framework of the Shimada classification with minor modifications, is proposed for international use in assessing NTs. *Cancer* 1999;86:364–72.

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A cooperative international effort has been in progress, assessing clinical and biologic characteristics of neuroblastic tumors (NTs) (a group of tumors including neuroblastoma, ganglioneuroblastoma, and ganglioneuroma) for the development of treatment strategies based on a complete set of International Neuroblastoma Risk Groups. The international collaboration to date has produced an International Neuroblastoma Staging System and a set of International Neuroblastoma Response Criteria.¹ The International Neuroblastoma Pathology Committee (INPC), established in 1994, has been actively participating in this effort by proposing a pathology classification for international use.

NTs once were described as “enigmatic” by many oncologists and

investigators because of their “unexpected” clinical behaviors, such as involution/spontaneous regression, maturation, and aggressive progression. Recent advances in clinical as well as basic research have been successfully accumulating data that are allowing us to describe NTs in a new way (i.e., NTs are “heterogeneous” with their individual biologic properties related closely to their unique clinical behavior²).

Historically, the principal morphologic feature recognized to be of prognostic importance for NTs is the degree of neuroblastic maturation toward ganglion cells.^{3,4} A number of histopathologic grading systems of NTs have been proposed in the past decades, yet to our knowledge not one has gained universal favor and application.^{5,6} Shimada et al.⁷ took a new approach with their age-linked classification, which divided NTs into Schwannian stroma-rich and stroma-poor tumors. They also have introduced the term “mitosis-karyorrhexis index” (MKI) for describing one of the prognostic indicators. Some modifications in this classification were published by Joshi et al.,^{8,9} who suggested a high mitotic rate to be an unfavorable prognostic factor and tumor-associated calcification to be a favorable prognostic factor. It has become apparent over the past decade that there are important biologic attributes of neuroblastic tumors that have impacted on our understanding of these neoplasms. The challenge of any pathology classification, in common with others in the past, is to formulate a reproducible and biologically meaningful system.

This report, based on the first international collaboration on NTs, summarizes 4-year activities of the INPC: i.e., 1) making consensus diagnoses according to uniform criteria of morphologic features and 2) testing the prognostic significance of the morphologic features and their combination by using the consensus diagnoses. The goals and objectives of the INPC are to propose an International Neuroblastoma Pathology Classification, which should be prognostically significant, biologically relevant, and at the same time highly reproducible and user friendly. Detailed criteria of morphologic features on the NTs used in this study have been summarized in a previous article.¹⁰ In the current study we also present a recommendation/guideline for surgical pathologists to use in their description and prognostic evaluation of the NTs.

MATERIALS AND METHODS

Materials

The patients used in the analysis were a subset of patients registered by January 8, 1995 in two Children’s Cancer Group studies: CCG-3881 (a study for low and intermediate risk neuroblastoma) and CCG-

3891 (a study for high risk neuroblastoma). Therefore this combined group of patients incorporates all risk groups. At the time that sample was created, a total of 535 patients from the two studies were identified as having pathology material available for review. A random sample of 152 patients (28% of these 535 patients) was taken, and, in addition, 75 subjects who had developed disease recurrence or died by January 1995 were added to the sample for a total of 227 patients in the analysis (150 patients from CCG-3881 and 77 patients from CCG-3891). This sampling scheme commonly is used in epidemiologic studies and is known as the “case-cohort” design.¹¹ It was used here to maximize the amount of information available in the statistical analysis, while keeping the total number of subjects limited to a number that could be reviewed over a limited period of time (all subjects were reviewed in 5 working days). Pathology slides of these 227 cases were collected from the CCG Neuroblastoma Pathology Repository, Childrens Hospital Los Angeles, Los Angeles, California, and randomly numbered for the review. The individual cases had varying numbers of sections (range, 1–35 sections, average 6 sections per case). The surgical pathology reports from the contributing institutions were carefully reviewed centrally by the pathologist of record (H.S.) to determine the eligibility of the cases for the study. Among these cases, 16 patients received intensive treatment with autologous bone marrow transplantation (ABMT) prior to the time of analysis of this study. The neuroblastoma study group for CCG currently is in the process of analyzing the results. There was a significant difference in event free survival (EFS) between ABMT and chemotherapy of slightly <15% for the highest risk group. This level of difference in so few patients treated with ABMT did not affect our results to any significant degree in this study.

Among these cases, 108 patients were age <1 year and 119 were age ≥1 year at the time of diagnosis. There were 30 Stage 1 cases, 59 Stage 2 cases, 31 Stage 3 cases, 87 Stage 4 cases, and 20 Stage 4-S cases included in this study. Forty-two tumors had amplified *MYCN*, 152 had nonamplified *MYCN*, and the *MYCN* test was not performed in 33 cases.

Pathology Review

Morphologic features of NTs to be evaluated by the review were determined after the first meeting in Los Angeles, California in 1994. They were defined precisely and described in a previous article.¹⁰ The pathology review was a step-wise evaluation of morphologic features without knowledge of clinical information. First, tumors were classified into four different categories: neuroblastoma (Schwannian stroma-poor); ganglioneuroblas-

toma, intermixed (Schwannian stroma-rich); ganglioneuroma (Schwannian stroma-dominant); and ganglioneuroblastoma, nodular (composite Schwannian stroma-rich/stroma-dominant and stroma-poor). Second, for neuroblastoma and the Schwannian stroma-poor area of ganglioneuroblastoma, nodular, the grade of neuroblastic differentiation (undifferentiated, poorly differentiated, and differentiating) was determined along with the other specific features (3 classes for MKI [low, intermediate, and high], two classes for mitotic rate [MR] [low and high], and the presence of calcification [yes or no]). Third, for ganglioneuroma, two subtypes (maturing and mature) were distinguished.

The first review session for the pathology slides from the 227 cases was performed by the 6 pathologists (H.S., I.M.A., L.P.D., J.H., V.V.J., and B.R.) individually in Oslo, Norway in 1995. After clarifying details regarding the morphologic features, the second review session was held in Tokyo, Japan in 1996. The second session was a group review, and the 5 pathologists present (H.S., I.M.A., J.H., V.V.J., and B.R.) examined the same case at the same time by using a multihead microscope. After review and discussion of the individual cases by the group review, each pathologist cast a vote for his or her final evaluation regarding type, subtype, grade of differentiation, MKI, MR, and calcification of the given tumor tissue. One pathologist (L.P.D.) was unable to participate in the group review.

Prognostic Analysis

A total of six diagnoses (including five diagnoses made by the group review and one diagnosis made by individual review [L.P.D.]) for each morphologic feature were collected. Consensus diagnoses for the individual morphologic features were decided by five of six or six of six agreements by the reviewer pathologists, and used for the analysis of the prognostic significance.

Prognostic effects of the individual morphologic features and prognostic groupings according to various classification systems were analyzed. The classification systems analyzed in this study were: 1) the Shimada classification⁷ (the favorable histology group, including: (a) neuroblastoma with low or intermediate MKI diagnosed at age <1.5 years, (b) neuroblastoma, differentiating subtype with low MKI diagnosed between ages 1.5–5 years, (c) ganglioneuroblastoma, intermixed in any age, and (d) ganglioneuroma in any age; and the unfavorable histology group, including: (a) neuroblastoma with high MKI in any age; (b) neuroblastoma, undifferentiated and poorly differentiated subtype diagnosed between ages 1.5–5 years; (c) neuroblastoma with intermediate MKI diagnosed between ages 1.5–5 years; (d) neuroblastoma diagnosed at age >5 years; and (e) ganglioneuroblastoma, nodular di-

agnosed at any age); 2) histologic grade⁸ (Grade 1: tumors with “MR = low and calcification = yes”; Grade 2: tumors with “MR = low and calcification = no” and “MR = high and calcification = yes”, and Grade 3: tumors with “MR = high and calcification = no”); 3) risk group⁸ (low risk: all Grade 1 tumors and Grade 2 tumors diagnosed at age > 12 months and high risk: all Grade 3 tumors and Grade 2 tumors diagnosed at age ≥ 12 months); 4) modified histologic grade¹² (modified Grade 1: tumors with “MKI = low or intermediate and calcification = yes,” modified Grade 2: tumors with “MKI = low or intermediate and calcification = no” and “MKI = high and calcification = yes,” modified Grade 3: tumors with “MKI = high and calcification = no”); and 5) modified risk group¹² (modified low risk: all modified Grade 1 tumors and modified Grade 2 tumors diagnosed at age < 12 months and modified high risk: all modified Grade 3 tumors and modified grade 2 tumors diagnosed at age ≥ 12 months). Prognostic evaluations by the histologic grade and the risk group were applicable only to the neuroblastoma (Schwannian stroma-poor) tumors.

Prognostic effects of the individual features and the prognostic groups according to the classification systems described earlier were analyzed. EFS was used as the endpoint in the analysis. Kaplan–Meier analysis (appropriately modified for the case–cohort selection) was used to produce estimates of the probability of EFS as specific times from diagnosis. Multivariate analysis of the joint significance of individual factors and prognostic groups was performed by the modified Cox regression technique.¹¹ All *P* values reported were the result of two-tailed tests. Chemotherapy differed between the two studies and by risk groups defined by stage and biology. Low risk patients (Stages 1, 2, and 4-S) generally received treatment with surgery alone. Intermediate risk patients (biologically favorable Stage 3 and Stage 4 infants) received a course of moderate therapy on CCG-3881 and high risk patients (biologically unfavorable Stage 3 or Stage 4 infants or Stage 4 patients age > 1 year) received intensive chemotherapy and possibly ABMT.

RESULTS

Among the 227 cases, consensus diagnoses for categorization were reached for 224 tumors (99%). There were 190 neuroblastoma (Schwannian stroma-poor) tumors; 5 ganglioneuroblastoma, intermixed (Schwannian stroma-rich) tumors; 1 ganglioneuroma (Schwannian stroma-dominant), maturing tumor; 19 ganglioneuroblastoma, nodular (composite Schwannian stroma-rich/stroma-dominant and stroma-poor) tumors; and 9 NT, unclassifiable cases (Table 1). Morphologic features of

TABLE 1
Categorization of 227 Neuroblastic Tumors by 6 Reviewer Pathologists

Categories	No. of cases
Consensus diagnosis (99%) ^a	
Neuroblastoma (Schwannian stroma-poor)	190
Ganglioneuroblastoma, intermixed (Schwannian stroma-rich)	5
Ganglioneuroma (Schwannian stroma-dominant)	1
Ganglioneuroblastoma, nodular (composite Schwannian stroma-rich/stroma-dominant and stroma-poor)	19
NT, unclassifiable ^b	9
Disagreement (1%)	3

NT: neuroblastic tumor.

^a Consensus diagnosis: based on five of six or six of six agreements by the reviewer pathologists.^b Criteria presented previously.¹⁰**TABLE 2**
Morphologic Features of 190 Cases of Neuroblastoma (Schwannian Stroma-Poor) by 6 Reviewer Pathologists

Neuroblastoma (Schwannian stroma-poor)	No. of cases
Subtype	
Consensus diagnosis (93%) ^a	
Undifferentiated	6
Poorly differentiated	155
Differentiating	15
Disagreement (7%)	14
MKI	
Consensus diagnosis (90%) ^a	
Low (< 100/5000)	94
Intermediate (100–200/5000)	40
High (> 200/5000)	37
Disagreement (10%)	19
Mitotic rate	
Consensus diagnosis (75%) ^a	
Low (≤ 10/10 HPF)	89
High (> 10/10 HPF)	50
Not determined	4
Disagreement (25%)	47
Calcification	
Consensus diagnosis (100%) ^a	
Yes	110
No	80
Disagreement (0%)	0

MKI: mitosis-karyorrhexis index; HPF: high-power fields.

^a Consensus diagnosis: based on five of six or six of six agreements by the reviewer pathologists.

the 190 neuroblastoma tumors are summarized in Table 2: 93% (176 of 190) had consensus diagnoses for their subtyping based on grade of neuroblastic differentiation (6 undifferentiated, 155 poorly differentiated, and 15 differentiating tumors). 90% (171 of 190) had consensus diagnoses for MKI (94 low MKI tumors, 40 intermediate MKI tumors, and 37 high MKI tumors). Approximately 75% (143 of 190) had consensus diagnoses for MR (89 low MR tumors and 50 high MR tumors), and 100% (190

TABLE 3
Prognostic Effects of the Morphologic Features in Neuroblastoma (Schwannian Stroma-Poor) Tumors

Morphologic features	Survival ^a	P value
Subtypes		
Undifferentiated (a)	30%	(a) vs. (b), $P = 0.11 \times 10^{-4}$
Poorly differentiated (b)	73%	(a) vs. (c), $P = 0.96 \times 10^{-4}$
Differentiating (c)	77%	(b) vs. (c), $P = 0.81$
MKI		
Low (a)	82%	(a) vs. (b), $P = 0.0069$
Intermediate (b)	65%	(a) vs. (c), $P = 0.77 \times 10^{-9}$
High (c)	23%	(b) vs. (c), $P = 0.026$
Mitotic rate		
Low	83%	$P = 0.0019$
High	58%	
Calcification		
Yes	76%	$P = 0.019$
No	62%	

MKI: mitosis-karyorrhexis index.

^a Survival is shown as expected 3-year progression free survival.

of 190) had consensus diagnoses for calcification (yes, 110 cases and no, 80 cases). With regard to the morphologic features of the stroma-poor portion of 19 ganglioneuroblastoma, nodular tumors, 95% (18 of 19) had consensus diagnoses for subtyping (12 poorly differentiated and 5 differentiated tumors with 1 not evaluable case); 95% (18 of 19) had consensus diagnoses for MKI (14 low MKI tumors, 2 intermediate MKI tumors, and 2 high MKI tumors); 89% (17 of 19) had consensus diagnoses for MR (14 low MR tumors and 1 high MR tumor with 2 not evaluable cases); and 100% (19 of 19) had consensus diagnoses for calcification (yes, 15; no, 3; and one not evaluable case).

Among these patients, 117 had events during their clinical course (follow-up period range, 0.68–57.87 months; median, 25.11 months); 42 showed disease progression but were alive at last follow-up, 66 showed disease progression and died of tumor, and 9 died of tumor soon after diagnosis. Prognostic significance of the individual morphologic features for the neuroblastoma tumors based on the consensus diagnoses is listed in Table 3. Nine cases of NT, unclassifiable were excluded from the prognostic analysis. The individual features had prognostic effects in EFS rates, with various degrees of significance. It was noted that those tumors of undifferentiated subtype had a significantly lower 3-year EFS of 30%. In addition, those tumors with high MKI showed a significantly lower 3-year EFS of only 23%. MR had the lowest consensus rate and did not add any significant prognostic information to that of MKI.

Because the number of cases of ganglioneuroblastoma, nodular was limited, analysis of prognostic ef-

TABLE 4
Prognostic Effects by Grouping

Prognostic grouping	Survival ^a	P value
Shimada classification (consensus ^b 90%)		
Favorable histology (N = 103)	85%	$P = 0.31 \times 10^{-9}$
Unfavorable histology (N = 93)	41%	
Histologic grade (consensus ^b 73%)		
Grade 1 (a) (N = 62)	84%	(a) vs. (b), $P = 0.12$
Grade 2 (b) (N = 57)	73%	(a) vs. (c), $P = 0.0088$
Grade 3 (c) (N = 20)	58%	(b) vs. (c), $P = 0.18$
Risk group (consensus ^b 73%)		
Low risk (N = 85)	85%	$P = 0.41 \times 10^{-3}$
High risk (N = 54)	59%	
Histologic grade, modified (consensus ^b 90%)		
Grade 1 (a) (N = 85)	78%	(a) vs. (b), $P = 0.36$
Grade 2 (b) (N = 63)	70%	(a) vs. (c), $P = 0.39 \times 10^{-6}$
Grade 3 (c) (N = 23)	18%	(b) vs. (c), $P = 0.13 \times 10^{-4}$
Risk group, modified (consensus ^b 90%)		
Low risk (N = 116)	80%	$P = 0.53 \times 10^{-5}$
High risk (N = 55)	42%	

The Shimada Classification was applied to all evaluable neuroblastic tumors (N = 218). Histologic grade; risk group; histologic grade, modified; and risk group, modified were applied only to the neuroblastoma (Schwannian stroma-poor) tumors (N = 190).

^a Survival is shown as the expected 3-year event free survival.

^b Consensus was based on five of six or six of six agreements by the reviewer pathologists.

fects for the individual morphologic features of their Schwannian stroma-poor portion was not performed. Two patients with ganglioneuroblastoma, intermixed had progression of disease after incomplete resection, but all 5 patients in this category and 1 patient with ganglioneuroma, maturing were alive after follow-up periods ranging from 1 year and 8.5 months to 4 years and 8 months.

Impacts on EFS rates by the different prognostic classification systems (i.e., the Shimada classification [consensus diagnoses for 90% cases], histologic grade [consensus diagnoses for 73% cases], risk group [consensus diagnoses for 73% cases], modified histologic grade [consensus diagnoses for 90% cases], and modified risk group [consensus diagnoses for 90% cases]) are summarized in Table 4. All the classifications had prognostic effects of various significance. Among them, the Shimada classification (age, categorization by Schwannian stromal development, subtyping by grade of neuroblastic differentiation, and MKI) and modified risk group (age, calcification, and MKI), had a very strong impact on the EFS rates of the patients with NTs, and showed very high consensus rates. Multivariate analysis disclosed that 1) the Shimada classification (Fig. 1) (103 tumors in the favorable histology group and 93 tumors in the unfavorable histology group) was the strongest and could add more prog-

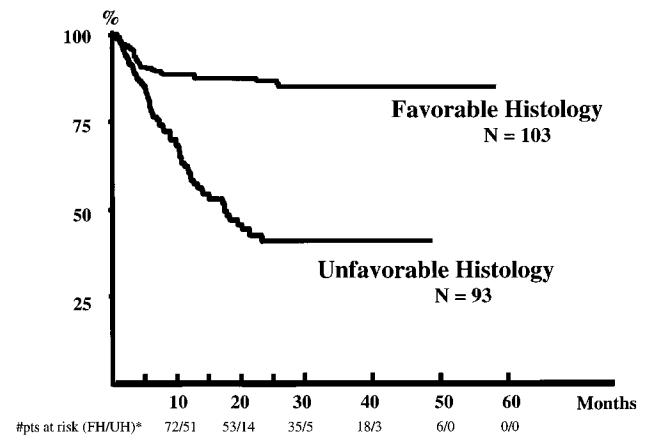


FIGURE 1. Kaplan–Meier curves (event free survival rates) for tumors with favorable and unfavorable histology according to the Shimada System ($P = 0.31 \times 10^{-9}$). Three-year 95% confidence intervals for favorable histology tumors were 0.7271, 0.9771 and those for tumors with unfavorable histology were 0.1901, 0.6313. *#pts. at risk (FH/UH): numbers of patients at risk at different time points, FH: favorable histology; UH: unfavorable histology.

nostic information to the prognostic effect of the modified risk group ($P = 0.0012$); 2) “calcification,” one of the prognostic indicators in the modified risk group, did not add any significant prognostic information to the Shimada classification; and 3) the prognostic effect of the modified risk group was influenced significantly by MKI alone.

Additional analyses were performed for the significance of age factor on the Shimada classification system. First, the system without age factor gave significantly lower prognostic effects than the original classification ($P = 0.54 \times 10^{-3}$). Then, Kaplan–Meier curves generated by different, month-by-month, age cutoffs from 12 months to 24 months demonstrated that the cutoff at 18 months of age used in the original classification gave the most significant information for prognostic evaluation. For example, P values indicating better prognostic effect of the cutoff at 18 months by direct comparison with other months were: 0.63×10^{-9} for 12 months versus 18 months, 0.052 for 14 months versus 18 months, 0.072 for 16 months versus 18 months, 0.076 for 20 months versus 18 months, 0.019 for 22 months versus 18 months, and 0.11 for 24 months versus 18 months.

Because undifferentiated subtype of neuroblastoma had a very low EFS rate, all six cases were listed in Table 5. It was noted that all cases were classified into an unfavorable histology group according to the Shimada classification system.

Table 6 shows distribution of age, clinical stage, and *MYCN* status by the International Neuroblastoma Pathology Classification (Shimada system; a total of

TABLE 5
List of 6 Cases with Undifferentiated Subtype of Neuroblastoma (Schwannian Stroma-Poor)

Age (mos)	MKI	Mitotic rate	Calcification	Shimada classification
17	High	NCD	No	Unfavorable
15	High	NCD	No	Unfavorable
4	High	NCD	No	Unfavorable
24	Intermediate	Low	No	Unfavorable
10	High	High	Yes	Unfavorable
48	Low	Low	No	Unfavorable

MKI: mitosis-karyorrhexis index; NCD: no consensus diagnosis.

TABLE 6
Prognostic Groups According to the International Neuroblastoma Pathology Classification: Case Distribution by Nonmorphologic Prognostic Factors

	International Neuroblastoma Pathology Classification (Shimada System)		P value
	Favorable histology	Unfavorable histology	
Age			
< 1 year	72	22	
≥ 1 year	31	71	< 0.0001
Clinical stage			
1 + 2 + 4-S	73	20	
3 + 4	30	73	< 0.0001
MYCN ^a			
Nonamplified	84	48	
Amplified	6	32	< 0.0001

^a MYCN status was tested in 170 of 196 available cases.

196 cases available for the analysis). It was apparent that the tumors in the favorable histology group were associated significantly with biologically favorable factors such as younger age at diagnosis (< 1 year), nonadvanced clinical stage (Stage 1 or 2) or Stage 4-S, and nonamplified *MYCN*, whereas the tumors in the unfavorable histology group frequently presented with biologically unfavorable factors such as older age at diagnosis (≥ 1 year), advanced clinical stage (Stage 3 or 4), and amplified *MYCN*.

DISCUSSION

Based on the detailed definition of morphologic features¹⁰ and the statistical analysis of pathology review data, the International Neuroblastoma Pathology Classification is proposed by adopting the Shimada classification system⁷ with minor modifications (Table 7). This is an age-linked classification system that prognostically is the most significant and biologically relevant. The Shimada system offers significantly bet-

ter prognostic information than the modified risk group¹² whose prognostic effect largely is influenced by MKI alone. The Committee members were able to produce consensus diagnoses in 90% of cases in the classification with very high levels of concordance of the individual morphologic features. Although the current study was not designed to test reproducibility, description of the criteria reported in a preceding article¹⁰ would assure pathologists, as it did the Committee members, a high consensus/concordance in making histologic evaluation of NTs. The Committee currently is planning to make an atlas of NT histopathology for international use. The International Neuroblastoma Pathology Classification is to serve a standardized formula of prognostic assessment by pathology evaluation of the NTs. The classification also can be used as a tool for comparison of histologic features with genetic and molecular-biologic properties of the tumors.

Biologic relevance of the morphologic features used in the International Neuroblastoma Pathology Classification, such as Schwannian stromal development in the age-linked maturational sequence of the NTs, nodular formation in the ganglioneuroblastoma, nodular (composite Schwannian stroma-rich/stroma-dominant and stroma-poor) tumors, and grade of neuroblastic differentiation and MKI in the neuroblastoma (Schwannian stroma-poor) tumors, has been discussed in a previous article.¹⁰ It should be noted again here that there is a reproducible correlation between a high MKI and adverse clinical and biologic (*MYCN* amplification) manifestations.¹³ It is speculated that the karyorrhectic process in tumor cells with amplified *MYCN* could be different biologically from the apoptotic process in those with potential for disease regression.¹⁰ Consensus rate and prognostic impact of the MR were not sufficient to be included in the prognostication. Calcification, in spite of its 100% consensus, did not appear to have an isolated prognostic impact.

The results of statistical analyses clearly demonstrated that age is critical in the prognostic evaluation of NT morphology. According to the Shimada system, there are two cutoffs for the age factor in neuroblastoma tumors: the prognostic significance of the cutoff age of 1.5 years was shown in this study, and that of another age cutoff of 5 years was confirmed by a recent study using a large series of cases.¹⁴ It becomes evident that two morphologic indicators (i.e., grade of neuroblastic differentiation and MKI) in neuroblastoma tumors have different prognostic effects depending on the patient's age at diagnosis. The data also support hypotheses used in the Shimada system: 1) neuroblastic cells with a maturational potential re-

TABLE 7
Prognostic Evaluation of Neuroblastic Tumors According to the International Neuroblastoma Pathology Classification (Shimada System)

International Neuroblastoma Pathology classification		Original Shimada classification	Prognostic group
Neuroblastoma	(Schwannian stroma-poor) ^a	Stroma-poor	
Favorable		Favorable	Favorable
< 1.5 yrs	Poorly differentiated or differentiating & low or intermediate MKI tumor		
1.5-5 yrs	Differentiating & low MKI tumor		
Unfavorable		Unfavorable	Unfavorable
< 1.5 yrs	a) undifferentiated tumor ^b		
	b) high MKI tumor		
1.5-5 yrs	a) undifferentiated or poorly differentiated tumor		
	b) intermediate or high MKI tumor		
≥5 yrs	All tumors		
Ganglioneuroblastoma, intermixed	(Schwannian stroma-rich)	Stroma-rich Intermixed (favorable)	Favorable ^c
Ganglioneuroma	(Schwannian stroma-dominant)		
Maturing		Well differentiated (favorable)	Favorable ^c
Mature		Ganglioneuroma	
Ganglioneuroblastoma, nodular	(composite Schwannian stroma-rich/stroma-dominant and stroma-poor)	Stroma-rich nodular (unfavorable)	Unfavorable ^c

MKI: mitosis-karyorrhexis index.

^a Subtypes of neuroblastoma were described in detail elsewhere.¹⁰^b Rare subtype, especially diagnosed in this age group. Further investigation and analysis required.^c Prognostic grouping for these tumor categories is not related to patient age.

quire an *in vivo* latent period for their demonstration of histologic evidence of differentiation and 2) there is a certain allowance for mitotic and karyorrhectic activities of neuroblastic cells in infants and younger children. Recent data suggest a biologically significant correlation between higher expression of nerve growth factor receptors and an age-linked morphologic differentiation of the neuroblastic cells in NTs.¹⁵

As illustrated in Table 7, the International Neuroblastoma Pathology Classification distinguishes favorable and unfavorable histology groups according to the Shimada system.⁷ A conceptual framework of age-linked maturation is used for identifying tumors in the former group, although tumors in the latter group have morphologic indicators suggesting their aggressive growth. Tumors in the favorable histology group are within an age-linked maturational sequence from poorly differentiated (age < 1.5 years) to differentiating (age < 5 years) neuroblastoma to ganglioneuroblastoma, intermixed and to ganglioneuroma. The neuroblastoma tumors in this group should have low (age < 5 years) or up to intermediate (age < 1.5 years) MKI. By contrast, tumors in the unfavorable histology group have immature histologies for the age of the patients and are classified into an undifferentiated (in any age) or poorly differentiated subtype (age ≥ 1.5 years) or any subtype (age ≥ 5 years) of the neuroblas-

toma. Among the neuroblastoma tumors, those with high MKI (in any age) or intermediate (age ≥ 1.5 years) also qualify as having an unfavorable histology. Neuroblastic nodular formation in the ganglioneuroblastoma, nodular tumor also is considered a sign of aggressive growth.

As shown in Table 6, tumors in the favorable histology group were associated with biologically favorable nonmorphologic prognostic factors, whereas tumors in the unfavorable histology group were related closely with biologically unfavorable, nonmorphologic prognostic factors. However, histopathologic evaluation according to the Shimada system has been shown to provide additional prognostic information beyond the factors of age, stage, and *MYCN* status.^{13,16}

The only differences between the original Shimada classification⁷ and the International Neuroblastoma Pathology Classification are 1) to subdivide the "undifferentiated" subtype in the former classification into two subtypes of undifferentiated and poorly differentiated in the latter classification, and 2) to change the name of "stroma-rich, well differentiated" in the former classification to "ganglioneuroma, maturing" in the latter classification. The undifferentiated subtype is created partly due to a practical reason¹⁰ and partly due to a possible prognostic implication: it contains a small number of cases in our series whose

prognosis was extremely poor. A similar poor prognosis has been reported in a previous series from the Pediatric Oncology Group (POG).⁹ All undifferentiated neuroblastoma tumors in our series, as well as a series from the POG, were classified into the unfavorable histology group according to the Shimada system. High MKI, another powerful indicator of poor prognosis among individual morphologic features, is alone a prognostic indicator for predicting aggressive tumor behavior in the Shimada system. Lastly, ganglioneuroma in the International Neuroblastoma Pathology Classification is accompanied by a term of Schwannian stroma-dominant in parenthesis.

Currently intergroup studies by CCG and POG in the North America are using the original Shimada classification for patient stratification and protocol assignment. We believe the International Neuroblastoma Pathology Classification (Shimada system) will become one of the official prognostic indicators for analyzing a large number of cases in their studies after the publication. The prognostic significance of the classification also will be tested in European countries as well as in Japan.

Guidelines for Description in the Surgical Pathology Report

Following are recommendations for the surgical pathologist in preparing pathology reports on NT.

The morphologic description of the NT should include information regarding the amount of Schwannian stroma and the presence or absence of macroscopic nodularity, indicating a diagnosis of composite tumor. The degree of neuroblastic differentiation and MKI are documented for the neuroblastoma and for the stroma-poor portion of the ganglioneuroblastoma, nodular. The description also includes a note regarding the presence or absence of calcification for future analysis (calcification is the only morphologic indicator used in the modified risk group¹² but is not included in the Shimada system⁷), but not for the current prognostic evaluation. It also should indicate the percentage of neuroblastic/ganglion cells versus Schwannian and other normal cells in the sample tissue(s) used for genetic/biologic studies (see recommendations for tissue handling and tissue/cell preservation in the article by Shimada et al.¹⁰).

The pathologic diagnosis includes a morphologic description only, expressing the category and subtype of the NT. If tumor cells were observed beyond the surgical margin(s) of resection, it should be indicated in the diagnosis without a conclusion as to whether the residual tumor is microscopic or macroscopic.

In comments accompanying the diagnosis, prognostic evaluation of morphologic features is de-

scribed in conjunction with the patient's age. In this section, the morphologic diagnosis also needs to be discussed in relation to known data of biologic importance. Results of the genetic and molecular biology studies (i.e., *MYCN* status, chromosome 1p deletions, DNA content, etc.) can be included in an integrated prognostic evaluation. *MYCN* amplification and deletions at chromosome 1p both suggest an unfavorable prognosis.¹⁷⁻¹⁹

With regard to the prognostic evaluation based on a limited amount of tissue from a pathology specimen, careful discussion should be required with oncology team members including an oncologist, radiologist, and surgeon (see problematic cases for histologic evaluation in the article by Shimada et al.¹⁰). This is very important, especially when differential diagnoses include ganglioneuroblastoma, intermixed (Schwannian stroma-rich), ganglioneuroma (Schwannian stroma-dominant), and ganglioneuroblastoma, nodular (composite Schwannian stroma-rich/stroma-dominant and stroma-poor). Prognostic evaluation based on the review of a limited amount of specimen should be offered only when all the team members are convinced that the submitted specimen is representative of the characteristics of the entire tumor tissue. Even then, the report should indicate clearly that the prognostic evaluation was made based on a limited amount of tissue sample. With regard to neuroblastoma, tumor tissue containing at least 5000 viable neuroblastic cells from multiple microscopic fields for the assessment of MKI and grade of neuroblastic differentiation is required for prognostic evaluation.

REFERENCES

1. Brodeur GM, Pritchard J, Berthold F, Carlsen NLT, Castel V, Castleberry RP, et al. Revisions of the International Criteria for Neuroblastoma Diagnosis, Staging, and Response to Treatment. *J Clin Oncol* 1993;11:1466-77.
2. Brodeur GM, Castleberry RP. Neuroblastoma. In: Pizzo PA, Poplack DG, editors. Principles and practice of pediatric oncology. 3rd edition. Philadelphia: Lippincott-Raven Publishers, 1997:761-97.
3. Beckwith JB, Martin RF. Observations on the histopathology of neuroblastomas. *J Pediatr Surg* 1968;3:106-10.
4. Hughes M, Marsden HB, Palmer MK. Histologic patterns of neuroblastoma related to prognosis and clinical staging. *Cancer* 1974;34:1706-11.
5. Dehner LP. Classic neuroblastoma: histopathologic grading as a prognostic indicator, The Shimada System and its progenitors. *Am J Pediatr Hematol Oncol* 1988;10:143-54.
6. Joshi VV, Tsongalis GJ. Correlation between morphologic and nonmorphologic prognostic markers of neuroblastoma. *Ann N Y Acad Sci* 1997;824:71-83.
7. Shimada H, Chatten J, Newton WA, Sachs N, Hamoudi AB, Chiba T, et al. Histopathologic prognostic factors in neuroblastic tumors: definition of subtypes of ganglioneuroblastoma and an age-linked classification of neuroblastomas. *J Natl Cancer Inst* 1984;73:405-16.

8. Joshi VV, Cantor AB, Altshuler A, Larkin EW, Neill JSA, Shuster JJ, et al. Age-linked prognostic categorization based on a new histologic grading system of neuroblastomas: a clinical pathologic study of 211 cases from the Pediatric Oncology Group. *Cancer* 1992;69:2197-211.
9. Joshi VV, Cantor AB, Altshuler A, Larkin EW, Neill JSA, Shuster JJ, et al. Recommendation for modification of terminology of neuroblastic tumors and prognostic significance of Shimada classification: a clinicopathologic study of 213 cases. *Cancer* 1992;69:2183-96.
10. Shimada H, Ambros I, Dehner LP, Hata J, Joshi VV, Roald B. Terminology and morphologic criteria of neuroblastic tumors: recommendations by the International Neuroblastoma Pathology Committee. *Cancer* 1999;86:348-62.
11. Prentice RL. A case-cohort design for epidemiologic cohort studies and disease prevention trials. *Biometrika* 1986;73:1-11.
12. Joshi VV, Rao PV, Cantor AB, Altshuler G, Shuster JJ, Castleberry RP. Modified histologic grading of neuroblastoma by replacement of mitotic rate with mitosis karyoprhexis index: a clinicopathologic study of 223 cases from the Pediatric Oncology Group. *Cancer* 1996;77:1582-8.
13. Shimada H, Stram D, Chatten J, Joshi VV, Hachitanda Y, Brodeur GM, et al. Identification of subsets of neuroblastomas combined histopathologic and N-myc analysis. *J Natl Cancer Inst* 1995;87:1470-6.
14. Monforte-Munoz H, Kawakami T, Stram D, Gerbing MA, Matthay K, Lukens J, et al. Neuroblastoma in children over 5 years of age: recognition of a rare and aggressive subset with "unconventional" morphology. *Mod Pathol* 1997;10(1):4P.
15. Peters J, Miyake M, Seeger RC, Cai B, Yao D, Hong CM, et al. Splicing pattern of the high affinity nerve growth factor receptor (trkA) transcript in neuroblastomas: correlation with histopathology and clinical outcome. *Lab Invest* 1995;72(1):144A.
16. Matthay KK, Perez C, Seeger RC, Brodeur GM, Shimada H, Atkinson JB, et al. Successful treatment for stage III neuroblastoma based on prospective biologic staging: a Childrens Cancer Group Study. *J Clin Oncol* 1998;16:1256-64.
17. Brodeur GM. Molecular basis for heterogeneity in human neuroblastoma. *Eur J Cancer* 1995;31A(4):505-10.
18. Ambros PF, Ambros IM, Strehl S, Bauer S, Luegmayr A, Kovar H, et al. Regression and progression in neuroblastoma. Does genetics predict tumour behavior? *Eur J Cancer* 1995;31A(4):510-5.
19. Caron H, Van Sluis P, de Kraker J, Bokkerink J, Egeler M, Laureys G, et al. Allelic loss of chromosome 1p as a predictor of unfavourable outcome in patients with neuroblastoma. *N Engl J Med* 1996;334:225-30.