

Biologic Variables in the Outcome of Stages I and II Neuroblastoma Treated With Surgery as Primary Therapy: A Children's Cancer Group Study

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Purpose: To determine prospectively whether surgery alone is sufficient therapy for Evans stages I and II neuroblastoma and to define biologic and clinical features having prognostic potential for this group.

Patients and Methods: Between June 1989 and August 1995, 374 eligible children (age range, 0 to 18 years) with newly diagnosed stage I (n = 141) and stage II (n = 233) neuroblastoma were registered onto Children's Cancer Group trial 3881. Surgical resection was the only primary therapy except in cases with spinal cord compression, where radiation therapy was allowed. Event-free survival (EFS) and overall survival (OS) were analyzed by life-table methods according to clinical and biologic features.

Results: EFS and OS (mean \pm SE) for all stage I patients were 93% \pm 3.0% and 99% \pm 1.0%, respectively, compared with 81% \pm 4.0% and 98% \pm 2.0%, respectively, for stage II patients. The significantly higher recurrence rate among stage II patients was

managed successfully in 38 of 43 children with either surgery or multimodality treatment. There was one death among stage I patients and six among stage II. For stage II patients tumor *MYCN* gene amplification, unfavorable histopathology, an age greater than 2 years, and positive lymph nodes predicted a lower OS ($P < .05$).

Conclusion: Children with stages I and II neuroblastoma have 98% survival with surgery alone as primary therapy. Supplemental treatment was necessary in only 10% of stage I patients and 20% of stage II patients. In children with localized neuroblastoma, a subset of patients that are at higher risk for death can be defined as those with stage II disease who have tumor *MYCN* amplification or who are ≥ 2 years of age with either unfavorable histopathology or positive lymph nodes.

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NEUROBLASTOMA IS the most common extracranial solid tumor of childhood. Approximately 25% of children with newly diagnosed neuroblastoma present with nonmetastatic and localized disease.^{1,2} Patients with localized disease (Evans stages I and II) have excellent survival rates and require less treatment than those with advanced stage disease.³⁻⁷ However, a small percentage of these patients subsequently relapse and die of their disease. Several clinical and biologic features have been associated

with a poor outcome in neuroblastoma. These include tumor *MYCN* amplification,⁸⁻¹⁰ elevated serum ferritin (≥ 143 ng/mL),¹¹ unfavorable histopathology,¹² elevated serum neuron specific enolase,¹³ age greater than 1 year, distant skeletal metastasis, and bone marrow involvement detected by either conventional¹⁴ or immunocytologic¹⁵ techniques. These risk factors have been examined primarily in the advanced stages of neuroblastoma. The utility of these factors for predicting outcome in localized neuroblastoma is less clear. Studies of *MYCN* amplification have yielded conflicting results. Although most studies have demonstrated that *MYCN*-amplified tumors correlate with poor prognosis,⁸⁻¹⁰ localized neuroblastoma with *MYCN* amplification and favorable histology has been successfully treated with surgery alone.^{16,17} The purpose of this prospective study was (1) to determine if surgery alone is sufficient therapy for stage I and II neuroblastoma and (2) to define prospectively biologic and clinical features having prognostic potential for localized neuroblastoma to identify those tumors requiring more than surgery.

PATIENTS AND METHODS

Patient Population

All children with stages I and II neuroblastoma were registered onto Children's Cancer Group (CCG) study 3881 from June 1989 to August 1995. Eligibility criteria for CCG-3881 included all newly diagnosed

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stage I and stage II neuroblastoma in children 0 to 18 years of age, except for those stage II patients older than 1 year of age with tumor *MYCN* amplification. A single patient in this group was transferred to the CCG-3891 high-risk protocol and is excluded from survival analyses. Signed informed consent with appropriate local institutional human research board approval was obtained for all patients on this study.

Biologic Features

Ferritin was measured by radioimmunoassay at the treating institutional laboratory and reported as unfavorable if greater than or equal to 143 ng/mL and favorable if less than 143 ng/mL.¹¹ *MYCN* gene copy was considered amplified if there were more than 10 copies and was measured until 1993 by Southern analysis of gene copy number¹⁰ and, after 1993, by *MYCN* protein expression by semiquantitative polymerase chain reaction and immunoperoxidase stain in the CCG reference laboratory.¹⁸ Central pathology review was performed by Dr. Shimada.¹² The *MYCN* and the Shimada histopathologic classification were accepted for analysis only when performed by the central CCG reference laboratory.

Staging

Diagnostic evaluation included surgical staging and standard laboratory and imaging studies (skeletal survey or bone scan, ultrasound, computed tomography or magnetic resonance imaging, and urine catecholamines), as well as bilateral bone marrow aspirate, biopsy, and immunocytology (sensitivity, one tumor cell per 10⁵ nucleated bone marrow cells).¹⁵ Metaiodobenzylguanidine scan was obtained, if available, but not mandated because the majority of institutions did not have this modality when the protocol opened. Both Evans¹⁴ and INSS¹⁹ staging were confirmed centrally by reviewing all pathology and surgical reports. Central review was performed by three experienced surgeons and two oncologists, including review of data forms and operative, radiology, and pathology reports. Lymph nodes were considered positive by pathologic assessment whether or not they were attached to the primary tumor; however, for INSS staging, only nonadherent lymph nodes were used in the designation of INSS stage 2b. Treatment was assigned according to Evans stage because INSS staging was not yet in use when the study opened.

Treatment

All patients with stage I disease were treated initially with surgery alone. Recurrence of localized disease was treated surgically if the recurrent tumor could be fully resected. Otherwise, recurrent local or regional disease was treated with combination chemotherapy, with cisplatin, cyclophosphamide, doxorubicin, and etoposide, as defined for patients on the CCG-3881 protocol.²⁰ Persistent disease was managed with delayed surgery and, if necessary, local radiation. The management of patients progressing with metastatic disease was left to the discretion of the responsible physician.

All stage II patients without tumor *MYCN* amplification were treated initially with surgery alone unless there were signs of spinal cord compression, in which case osteoplastic laminotomy or radiation was recommended. Recurrence of localized disease in a patient with a stage II tumor was also managed surgically, if possible. Unresectable disease or regional progression was treated with combined-modality therapy, as described above. Patients who developed metastatic disease went off protocol therapy.

Stage II patients less than 1 year of age with tumor *MYCN* amplification were managed with combined-modality therapy, includ-

ing chemotherapy, as well as surgery to the residual disease and local radiation for gross residual disease after delayed surgery.²⁰ Patients with progressive disease were off protocol therapy. Stage II patients older than 1 year of age with tumor *MYCN* amplification, which included only one patient from this study, were excluded from CCG-3881 and registered onto CCG-3891. In this protocol, the same four chemotherapeutic agents from the CCG-3881 trial (described above) are used in a more dose-intensive induction, with subsequent randomization between high-dose consolidation therapy and myeloablative therapy with autologous purged bone marrow transplantation.²⁰

Statistical Analysis

Life-table methods were used to estimate the event-free survival (EFS) and overall survival (OS) from time of diagnosis.²¹ The exact test from the permutation distribution of the log-rank statistic was used to compare the OS probabilities between subgroups of patients.²² For all analyses of EFS and OS, the single patient with stage II disease, *MYCN* amplification, and age older than 1 year, who was transferred to CCG-3891, was excluded. Additionally, one death occurred unrelated to treatment or to disease process. This patient was censored 1 day before death.

RESULTS

Patient Characteristics

Clinical and biologic characteristics are listed in Table 1. A total of 374 patients with localized neuroblastoma, including stage I (n = 141) and stage II (n = 233), were registered onto this study. There was a single stage II patient transferred to CCG-3891 (>1 year of age with tumor *MYCN* amplification). All Evans stage I patients were also International Staging System for Neuroblastoma (INSS) stage 1; 102 stage II patients were INSS stage 1, 27 were INSS stage 2a, and 104 were INSS stage 2b. The median age at diagnosis was 10 months, with a range of 0 to 205 months. Forty-one percent of all patients had an abdominal primary site, and intraspinal tumor extension occurred in 65 stage II patients (28%) and in one stage I patient. Lymph node involvement was seen in 51% of stage II tumors (including adherent as well as nonadherent nodes).

Biologic characteristics with purportedly unfavorable prognostic implications were rare in this group of patients. Overall compliance with biologic factor evaluation was 92% for histopathology, 83% for *MYCN* gene-copy measurement, and 60% for bone marrow immunocytology. Elevated serum ferritin levels (≥ 143 ng/mL) were detected in 17% of patients tested. *MYCN* oncogene was amplified in only seven (2%) of 309 tumors. Shimada histopathology was unfavorable in 14% of the tumors evaluated. Bone marrow immunocytology showed tumor in only eight (4%) of 213 patients tested, all of whom had negative bone marrow aspirate and biopsy by standard light microscopy.

Table 1. Patient Characteristics

Characteristic	No. of Patients	
	Stage I (n = 141)	Stage II (n = 233)
Age		
< 2 years	116	180
≥ 2 years	25	53
Median, months	9	11
Range, months	205	0-178
Sex		
Male	83	106
Female	58	127
INSS stage		0-178
1	141	102
2a	0	27
2b	0	104
Intraspinal extension	1	65
Primary site		
Neck	6	17
Chest	50	118
Thoraco-abdominal	1	6
Adrenal	54	43
Celiac	1	2
Other abdominal	18	35
Pelvis	8	4
Other	3	8
Lymph nodes involved*		
Yes	0	114
No	140	109
Unknown	1	10
Ferritin ≥ 143†	19/24	30/195
MYCN amplified†	4/108	3/201
Shimada unfavorable†	16/128	32/216
BMI positive†‡	1/73	7/140

Abbreviation: BMI, bone marrow immunocytology.

* Includes any positive nodes, whether or not adherent to tumor.

† Number of patients/total number of patients with measurement.

‡ BMI = tumor cells per 10^5 nucleated bone marrow cells.

EFS, OS, and Prognostic Variables

The EFS for all stage I patients at 4 years was $93\% \pm 3\%$, with a median follow-up of 45 months (range, 0 to 88 months) (Fig 1A). Univariate analysis of risk factors for stage I disease demonstrated that *MYCN* amplification was the only factor associated with a decrease in EFS ($P < .001$). The 4-year OS for stage I patients was $99\% \pm 1\%$ (Fig 1B). Because there was only one death in this group, a 9-year-old patient whose tumor had unfavorable Shimada classification, one cannot be certain of the importance of prognostic factors in OS.

The EFS (mean \pm SE) for all stage II patients at 4 years was $81\% \pm 4\%$, and the OS was $98\% \pm 1.5\%$, with a median follow-up of 41 months (range, 0 to 92 months). Although EFS was significantly lower for stage II patients than stage I ($81\% v 93\%$, respectively; $P = .002$), there was no difference in the OS ($99\% v 98\%$, respectively) (Figs 1A

and 1B). Univariate analysis of risk factors for stage II disease demonstrated that female sex and intraspinal tumor extension were significantly associated with a decrease in EFS ($P < .05$) but not survival. Unfavorable histology was associated with a decrease both in EFS and OS ($P < .05$). Age ≥ 2 years was predictive of lower OS, but age ≥ 1 year was not a significant factor. *MYCN* amplification was associated with a significant decrease in OS (Table 2); lymph nodes were of borderline significance. After stratification by age ≥ 2 years, unfavorable histology ($P = .0005$) and positive lymph nodes ($P = .0024$) remained significant prognostic factors for OS only in those patients ≥ 2 years of age.

Local Control and Recurrences

Complete tumor resection was the therapeutic goal for localized disease. Gross, total tumor resection (complete

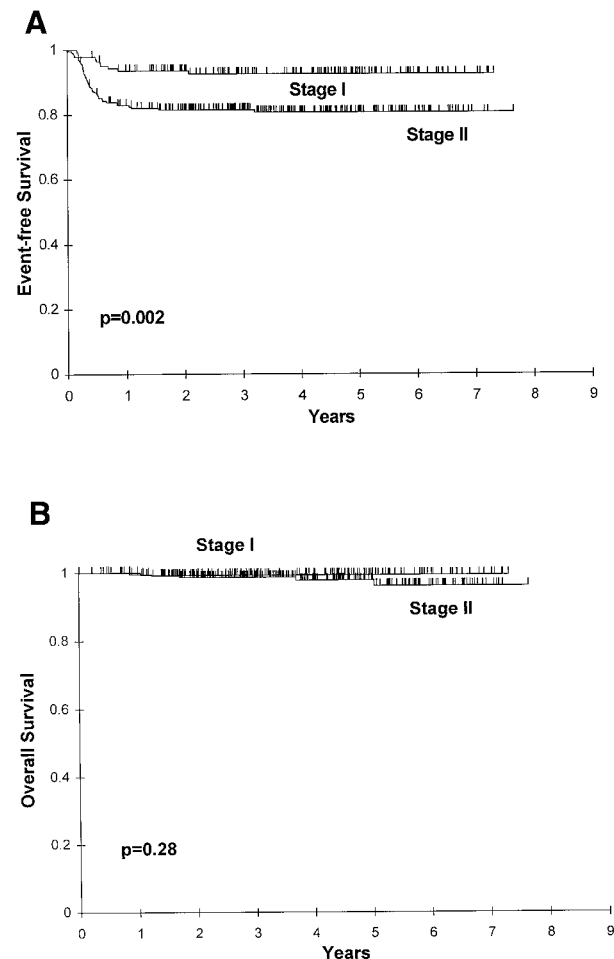


Fig 1. (A) EFS of children with stage I (n = 141) and stage II (n = 232) neuroblastoma ($P = .002$). (B) OS of children with stage I (n = 141) and stage II (n = 232) neuroblastoma ($P = .28$).

Table 2. Univariate Analysis of Risk Factors and OS for Stage II Patients

Risk Factor	A v B	4-Year OS				P
		A		B		
		%	SE	%	SE	
Age	< 2 yr v ≥ 2 yr	99	0.8	90	7.3	.0007
	< 1 yr v ≥ 1 yr	99	1.3	96	2.9	NS
Sex	Female v male	98	1.7	97	2.6	NS
Gross resection	Yes v no	97	1.6	100	0	NS
Intraspinal extension	Present v absent	97	3.3	98	98	NS
Primary site	Nonabdominal v abdominal	98	2.0	97	2.5	NS
Lymph nodes	Negative v positive	100	0	95	3.0	.054
MYCN gene	NA v A	98	1.6	50	35.4	.029
Histopathology	F v U	99	0.8	87	8.4	.0015
BMI	Negative v positive	96	2.5	100	0	NS
Ferritin, ng/mL	< 143 v ≥ 143	97	1.1	100	0	NS

Abbreviations: SE, standard error; NA, not amplified; A, amplified; F, favorable; U, unfavorable; BMI, bone marrow immunocytology (sensitivity, one tumor cell per 10⁵ nucleated cells); NS, not significant.

resection with negative surgical margins or microscopic residual disease) was achieved in all stage I tumors and, ultimately, in 90% of stage II tumors (Table 3). Only 23 patients had incomplete resection with residual gross disease. Extent of resection (complete gross resection v incomplete or partial resection) had no effect on EFS or OS ($P > .05$). All stage II patients with incomplete resection ($n = 23$) are alive, with follow-up ranging from 9 to 78 months. There have been seven recurrences in this group, five with primary or regional relapse only, one with bone metastasis, and one with local relapse and liver metastasis. All relapses were managed effectively using surgery alone or with multimodality therapy. Intraspinal extension occurred in 66 tumors, with neurologic symptoms in 20 patients. Of the symptomatic patients, 18 were treated only with surgery initially, two received chemotherapy, and none received radiation. Subsequent to diagnosis, five other patients received chemotherapy for persistent or progressive disease. However, six of the 46 asymptomatic patients were treated with chemotherapy alone and two with radiation and chemotherapy. Out of 66 patients with intraspinal involvement, there were 20 recurrences compared with only 23 out of 167

stage II patients without intraspinal involvement, although ultimate survival did not significantly differ.

Progression and Death in Stage I Patients

A total of 10 patients with stage I tumors developed progressive disease, including two patients with primary and distant relapse, four with metastatic disease only, and four with primary or regional only (Table 3). Six patients developed metastatic disease (three with bone metastasis, found at 2, 6, and 25 months; one with skin metastasis having an adrenal primary; one with abdominal metastasis having a chest primary; and one with distant lymph node metastasis). All but one patient survived. Of the remaining four patients with relapse involving the primary site, one was treated with a combination of surgery and chemotherapy, one with surgery alone, one with combined chemotherapy and radiation, and the fourth patient with chemotherapy alone. A single death occurred in a child 9 years of age at the time of diagnosis. The patient developed progressive disease and died of infectious complications. Patient characteristics for all deaths are listed in Table 4.

Progression and Death in Stage II Patients

A total of 43 patients with stage II tumors developed progressive disease, including 12 patients with primary and distant relapse, eight with distant only, and 23 with primary or regional only (Table 3). Twenty patients developed metastatic disease (eight of whom had bone metastasis). Of the 23 patients whose relapse involved only the primary site, 15 patients underwent a second operation (four with additional chemotherapy, one with chemotherapy and intraoperative radiation), seven were treated with chemotherapy alone, and a single patient was treated with both chemo-

Table 3. Local Control

	No. of Patients	
	Stage I (n = 140)	Stage II (n = 233)
Gross total resection at diagnosis	140	194
Overall gross total resection*	140	210
Total number of recurrences	10	43
Primary and distant	2	12
Distant only	4	8
Primary or regional only	4	23

* One patient missing data.

Table 4. Deaths

Age at Dx (months)	Sex	Evans Stage	Positive Lymph Nodes	INSS Stage	Best Resection Status	Primary Site	Shimada Histology	MYCN	Progression Site after DX	Cause of Death
112	M	I	No	1	CR	Adrenal	Unfavorable	Nonamplified	Bone/bone marrow	Infection
9	M	II	Yes	2b	MR	Adrenal	Favorable	Amplified	Liver	PD
38	M	II	Yes	2b	CR	Chest	Unfavorable	Nonamplified	Bone/bone marrow	PD
44	F	II	Yes	2b	MR	Chest	Unfavorable	Nonamplified	Bone/bone marrow	PD
31	F	II	Yes	2b	CR	Adrenal	Unfavorable	Nonamplified	Adrenal/bone/bone marrow	PD
28	F	II	Yes	2b	CR	Adrenal	Unfavorable	Nonamplified	Adrenal/bone/bone marrow	PD
37*	M	II	Yes	2b	CR	Adrenal	Unfavorable	Amplified	Adrenal/bone/bone marrow	PD

Abbreviations: Dx, diagnosis; PD, progressive disease; CR, complete resection; MR, microscopic residual; M, male; F, female.

* This patient was treated on CCG-3891 and, therefore, not included in EFS and OS analyses.

therapy and radiation. The time course to distant metastasis was relatively short, at a median of 4.5 months from diagnosis (range, 1.4 to 38.5 months). There were a total of six deaths, with all attributable to progressive disease (Table 4). There was one additional death that was unrelated to the disease process or treatment. This patient was censored 1 day before death so that the death is not included in our analysis of EFS and OS. Five of six deaths occurred in children older than 2 years of age. These five deaths were also associated with unfavorable histopathology, and one patient, treated on the CCG-3891 protocol, had tumor *MYCN* amplification. All six patients who died with stage II tumors had lymph node involvement (INSS stage 2b). One death occurred in a 9-month-old infant with *MYCN* amplification.

MYCN-Amplified Tumors

Seven patients had tumor *MYCN* amplification (> 10 copies), including three with stage II and four with stage I.

Details of the clinical course and outcome are listed in Table 5. Three of the seven patients had favorable Shimada classification, one of whom died. Four of the patients relapsed, and three died. Only one patient with stage I disease was successfully treated with surgery alone. The single death among the *MYCN*-amplified stage I patients was reported after initial submission of this manuscript and, therefore, was not included in the analyses of survival or in Table 4.

DISCUSSION

The purpose of the study was to determine prospectively whether surgery alone is sufficient therapy for localized neuroblastoma, with chemotherapy and radiation reserved for progression or recurrence of disease. A secondary purpose was to determine the prognostic potential of biologic and clinical variables of localized neuroblastoma. Of 141 stage I patients, there were only 10 relapses, which

Table 5. MYCN-Amplified Tumors

Age at Diagnosis (months)	Sex	Evans Stage	INSS Stage	Primary Site	Shimada Histology	Relapse		Comments
						Time from Diagnosis (months)	Site	
9	M	II	2b	Adrenal	Favorable	2	Liver	Died of PD 10 months after relapse; chemotherapy CCG-3881
5	M	II	2b	Adrenal	Unfavorable	None	–	Alive 80 months after Dx, chemotherapy CCG-3881, NED
37*	M	II	2b	Adrenal	Unfavorable	22	Bone marrow	Died of PD 8 months after relapse; CCG-3891
7	M	I	1	Adrenal	Favorable	6	Adrenal	Alive 17 months after three relapses, NED after CCG-3891, radiation, 13- <i>cis</i> -retinoic acid, desferal
10†	F	I	1	Adrenal	Unfavorable	None	–	Alive 67 months after Dx, chemotherapy CCG-3881, NED
4‡	M	I	1	Adrenal	Unfavorable	2	Bone; CNS	Died of PD after ABMT, 25 months post-relapse
5 days	F	I	1	Pelvis	Favorable	None	–	Alive 72 months after Dx, surgery only, NED

Abbreviations: PD, progressive disease; M, male; F, female; NED, no evidence of disease; Dx, diagnosis; ABMT, autologous bone marrow transplant.

* This patient was treated on CCG-3891 and, therefore, not included in EFS and OS analyses.

† Initially staged as Evans II. Patient received chemotherapy in addition to surgery.

‡ Died after analysis cutoff; therefore, not included in survival analyses or Table 4.

were managed either with surgery alone or with surgery with chemotherapy and/or radiation therapy. This approach resulted in only one death, for a 4-year EFS and OS of 93% and 99%, respectively. This result, for stage I disease (all INSS stage 1) again proves that surgery is sufficient therapy for almost all children with INSS stage 1, as previously shown for similar patients in other large studies.^{7,23-25} For 233 stage II patients, 43 had relapses, for a significantly lower EFS of 81%. However, OS was not significantly different at 98%, showing that a primary salvage treatment of surgery alone can be effective for patients with recurrent disease. In all, only 13% of children with stage II disease received chemotherapy or radiotherapy, despite the fact that 104 patients had INSS stage 2b. Previous large cooperative studies used primary chemotherapy to treat patients with positive lymph nodes or residual disease after surgery.²⁴⁻²⁶ It is possible that some of the early distant relapses were actually stage IV patients who were understaged at diagnosis because metaiodobenzylguanidine scan was not available at most institutions. This would result in even fewer progressions among the “true” stage I and II patients. Thus, we have shown that primary surgery as the sole therapeutic modality is sufficient therapy for approximately 90% of stage I patients and 82% of stage II patients, with excellent survival rates.

There was no difference in EFS and OS between stage II patients achieving gross, total primary tumor resection (complete resection or microscopic residual) and those with incomplete or partial resection. This is consistent with other reports from previous CCG studies⁶ and more recent reports from single institutions.^{5,23} The favorable outcome in most of these patients, despite some having gross residual disease, is consistent with the spontaneous regression or maturation of some forms of neuroblastoma. With this information, complex surgical procedures in low-stage disease to achieve complete resection should be avoided. This study provides strong evidence that the majority of stage I and II patients can be managed with surgery alone as primary therapy, even if complete gross resection is not feasible.

Prognostic Variables

Lymph node status as a prognostic factor in low-stage neuroblastoma has been an area of controversy. The prognostic value of involved lymph nodes is unclear; some studies show an adverse impact on outcome,^{24,27,28} whereas others find that positive nodes do not influence outcome.^{4-6,23} This study shows a small difference in survival for patients with stage II disease and positive nodes, which was only significant in patients greater than or equal to 2 years old at diagnosis. All six of the stage II deaths occurred in

patients having positive, nonadherent lymph nodes (INSS stage 2b). This was also associated with other unfavorable factors. Two of the patients had tumor *MYCN* amplification, and five had unfavorable histopathology; five of the six patients were older than 2 years of age (Table 4). Our findings suggest that lymph node metastasis in patients with other unfavorable clinical or biologic features may be a significant prognostic factor, and these children may ultimately require additional therapy. However, the rarity of events overall does not yet warrant adjuvant therapy for all such patients.

Age has long been known to be an independent prognostic variable.²⁹ Usually, patients less than 1 year of age have a more favorable outcome. However, in our study, survival was adversely affected for both stages I and II patients 2 years of age or older but not for those 1 year of age or older. A similar trend was seen for high-risk neuroblastoma in a previous study, where children between the ages of 1 and 2 years had a more favorable outcome than those older than 2 years at diagnosis.³⁰ Further analysis of the stage II patients demonstrated that those patients 2 years of age or older who relapsed, died more quickly of their disease ($P = .003$). The single death in stage I patients was a child 9 years of age with unfavorable histology. Five of the six deaths in stage II patients occurred in children older than 2 years of age with unfavorable histology.

The site of the primary tumor (abdominal v nonabdominal), sex, and intraspinal tumor extension were evaluated for prognostic significance. Previous studies have demonstrated the adverse prognostic significance of abdominal primaries^{31,32} and favorable outcome in tumors with intraspinal extension.^{33,34} In our study, abdominal primary was not associated with worse EFS or OS. On the other hand, intraspinal tumor extension had an adverse effect on EFS, although it did not influence survival. Sex seemed to have an impact on EFS, with stage II males having improved outcome. This has not been consistently reported and is of no prognostic significance in other studies.³⁵ There was no sex advantage offered for stage I patients, and OS was not significantly affected for either group by sex. Therefore, it is possible that this isolated effect on EFS is a result of random chance without true prognostic significance.

MYCN-amplified tumors have been linked to more aggressive behavior and rapid tumor progression, especially in advanced-stage neuroblastoma.⁸⁻¹⁰ The prognostic significance in low-stage neuroblastoma is unclear. Fabbretti et al¹⁶ reported two low-stage children with *MYCN*-amplified tumors and favorable Shimada histopathology, who were treated with surgery alone, alive and disease-free after 16 and 17 months follow-up. Cohn et al¹⁷ reported similar results in two patients with low-stage disease, *MYCN*

amplification, and favorable histology. These patients remained disease-free at 22+ and 16+ months, with no postoperative therapy. In contrast, patients with *MYCN* amplification and unfavorable histology progressed. It is difficult to draw definitive conclusions concerning the prognostic significance of *MYCN* amplification for low-stage tumor because of its rarity.^{16,17,25,36} In our study, only 2% of stage I and II tumors exhibited *MYCN* amplification with greater than 10 copies (four stage I and three stage II patients), with available data in 83% of tumors. All four stage I patients in our study with *MYCN* amplification were alive at the cutoff date for analysis, including two patients with favorable and two with unfavorable histology, although one subsequently died of disease. Of the three stage II patients with *MYCN* amplification, two died of progressive disease and one, with unfavorable histology, was alive and disease-free 80 months after diagnosis (Table 5). However, only one of all these patients could be treated with only surgery. *MYCN* amplification seems to be predictive of decreased survival only for stage II patients. However, given the small numbers, we cannot be certain about the importance of this finding. Additionally, no definitive conclusions can be made with respect to histologic differentiation and tumor progression in *MYCN*-amplified tumors.

Unfavorable Shimada histopathology is another independent factor associated with poor prognosis.¹² In our study, unfavorable histopathology was of prognostic significance for EFS and for OS in stage II tumors. The single death in the stage I patient and all five deaths in children with stage II tumors who were older than 2 years of age occurred in tumors having unfavorable histology, suggesting that unfavorable histology contributes to an unfavorable outcome.

Serum ferritin has been of prognostic significance in higher stage neuroblastoma.¹¹ Additionally, positive bone marrow immunocytology (> six tumor cells per 100,000 normal cells) was associated with poor outcome for stage II and III, but not stage I, disease.¹⁵ There were only one stage I and seven stage II patients with positive bone marrow immunocytology. Marrow disease remained quiescent in all patients with no evidence of progressive disease. In this study, neither elevated serum ferritin nor positive bone marrow immunocytology were of prognostic significance. Other biologic tumor characteristics currently under investigation may predict more precisely the ability of these low-stage tumors to metastasize, such as genetic aberrations recently reported to have prognostic value, including abnormalities at chromosomes 1p, 11q, 14q, and 17q or increased expression of telomerase.³⁷

In conclusion, we have shown that those with stages I and II neuroblastoma are a biologically favorable group of patients with excellent prognosis. Surgery alone is sufficient initial therapy for almost all patients, regardless of other clinical or biologic factors, with OS of 99% for stage I and 98% for stage II patients. The rarity of deaths in this study gives extremely limited power to look at combinations of risk factors for OS in multivariate analysis. *MYCN* was associated with decreased EFS for stage I, but because only a single death occurred, no factor could be associated with lower survival. There were statistically significant decreases seen in OS for stage II patients 2 years of age or older with unfavorable histology and *MYCN* amplification; positive nodes were of borderline significance. However, in view of the excellent salvage in most patients, multimodal therapy should be reserved for those who develop progressive disease.

APPENDIX

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REFERENCES

- Berthold F, Brandeis WE, Lampert F: Neuroblastoma: Diagnostic advances and therapeutic results in 370 patients. *Monogr Paediatr* 18:206-223, 1996
- Kinnier-Wilson LM, Draper GJ: Neuroblastoma, its natural history and prognosis: A study of 487 cases. *BMJ* 3:301-307, 1974
- Evans AR, Brand W, de Lorimier A, et al: Results in children with local and regional neuroblastoma managed with and without vincristine, cyclophosphamide, and imidazolecarboxamide: A report from the Children's Cancer Study Group. *Am J Clin Oncol* 7:3-7, 1984
- Hayes FA, Green A, Hustu HO, et al: Surgicopathologic staging of neuroblastoma: Prognostic significance of regional lymph node metastases. *J Pediatrics* 102:59-62, 1983
- Kushner BH, Cheung NK, LaQuaglia MP, et al: Survival from locally invasive or widespread neuroblastoma without cytotoxic therapy. *J Clin Oncol* 14:373-381, 1996
- Matthay KK, Sather HN, Seeger RC, et al: Excellent outcome of stage II neuroblastoma is independent of residual disease and radiation therapy. *J Clin Oncol* 7:236-244, 1989
- Nitschke R, Smith EI, Shochat S, et al: Localized neuroblastoma treated by surgery: A Pediatric Oncology Group study. *J Clin Oncol* 6:1271-1279, 1988
- Brodeur GM, Seeger RC, Schwab M, et al: Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science* 224:1121-1124, 1984
- Brodeur GM, Seeger RC, Sather H, et al: Clinical implications of oncogene activation in human neuroblastomas. *Cancer* 58:541-545, 1986
- Seeger RC, Brodeur GM, Sather H, et al: Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *N Engl J Med* 313:1111-1116, 1985
- Hann HW, Evans AE, Siegel SE, et al: Prognostic importance of serum ferritin in patients with Stages III and IV neuroblastoma: The Childrens Cancer Study Group experience. *Cancer Res* 45:2843-2848, 1985
- Shimada H, Chatten J, Newton WA Jr, et al: Histopathologic prognostic factors in neuroblastic tumors: Definition of subtypes of

ganglioneuroblastoma and an age-linked classification of neuroblastomas. *J Natl Cancer Inst* 73:405-416, 1984

13. Zeltzer PM, Marangos PJ, Evans AE, et al: Serum neuron-specific enolase in children with neuroblastoma: Relationship to stage and disease course. *Cancer* 57:1230-1234, 1986

14. Evans AE, D'Angio GJ, Randolph J: A proposed staging for children with neuroblastoma: Children's Cancer Study Group A. *Cancer* 27:374-378, 1971

15. Moss TJ, Reynolds CP, Sather HN, et al: Prognostic value of immunocytologic detection of bone marrow metastases in neuroblastoma. *N Engl J Med* 324:219-226, 1991

16. Fabbretti G, Valenti C, Loda M, et al: N-myc gene amplification/expression in localized stroma-rich neuroblastoma (ganglioneuroblastoma). *Hum Pathol* 24:294-297, 1993

17. Cohn SL, Look AT, Joshi VV, et al: Lack of correlation of N-myc gene amplification with prognosis in localized neuroblastoma: A Pediatric Oncology Group study. *Cancer Res* 55:721-726, 1995

18. Seeger RC, Wada R, Brodeur GM, et al: Expression of N-myc by neuroblastomas with one or multiple copies of the oncogene. *Prog Clin Biol Res* 271:41-49, 1988

19. Brodeur GM, Pritchard J, Berthold F, et al: Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment [see comments]. *J Clin Oncol* 11:1466-1477, 1993

20. Matthay KK, Perez C, Seeger RC, et al: Successful treatment of stage III neuroblastoma based on prospective biologic staging: A Children's Cancer Group study. *J Clin Oncol* 16:1256-1264, 1998

21. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 53:457-481, 1958

22. Kalbfleisch JD, Prentice RL: *The Statistical Analysis of Failure Time Data*. New York, NY, John Wiley and Sons, Inc, 1980

23. Evans AE, Silber JH, Shpilsky A, et al: Successful management of low-stage neuroblastoma without adjuvant therapies: A comparison of two decades, 1972 through 1981 and 1982 through 1992, in a single institution. *J Clin Oncol* 14:2504-2510, 1996

24. De Bernardi B, Conte M, Mancini A, et al: Localized resectable neuroblastoma: Results of the second study of the Italian Cooperative Group for Neuroblastoma. *J Clin Oncol* 13:884-893, 1995

25. Rubie H, Hartmann O, Michon J, et al: N-Myc gene amplification is a major prognostic factor in localized neuroblastoma: Results of

the French NBL 90 study—Neuroblastoma Study Group of the Societe Francaise d'Oncologie Paediatric. *J Clin Oncol* 15:1171-1182, 1997

26. Nitschke R, Smith EI, Altshuler G, et al: Postoperative treatment of nonmetastatic visible residual neuroblastoma: A Pediatric Oncology Group study. *J Clin Oncol* 9:1181-1188, 1991

27. Ninane J, Wese FX: Treatment of localized neuroblastoma. *Am J Pediatr Hematol Oncol* 8:248-252, 1986

28. Castleberry RP, Kun LE, Shuster JJ, et al: Radiotherapy improves the outlook for patients older than 1 year with Pediatric Oncology Group stage C neuroblastoma [see comments]. *J Clin Oncol* 9:789-795, 1991

29. Breslow N, McCann B: Statistical estimation of prognosis for children with neuroblastoma. *Cancer Res* 31:2098-2103, 1971

30. Stram DO, Matthay KK, O'Leary M, et al: Consolidation chemoradiotherapy and autologous bone marrow transplantation versus continued chemotherapy for metastatic neuroblastoma: A report of two concurrent Children's Cancer Group studies [see comments]. *J Clin Oncol* 14:2417-2426, 1996

31. Garaventa A, De Bernardi B, Pianca C, et al: Localized but unresectable neuroblastoma: Treatment and outcome of 145 cases. *J Clin Oncol* 11:1770-1779, 1993

32. Bowman LC, Castleberry RP, Cantor A, et al: Genetic staging of unresectable or metastatic neuroblastoma in infants: A Pediatric Oncology Group study. *J Natl Cancer Inst* 89:373-380, 1997

33. Massad M, Haddad F, Slim M, et al: Spinal cord compression in neuroblastoma. *Surg Neurol* 23:567-572, 1985

34. Plantaz D, Hartmann O, Kalifa C, et al: Localized dumbbell neuroblastoma: A study of 25 cases treated between 1982 and 1987 using the same protocol. *Med Pediatr Oncol* 21:249-253, 1993

35. Berthold F, Kassenbohmer R, Zieschang J: Multivariate evaluation of prognostic factors in localized neuroblastoma. *Am J Pediatr Hematol Oncol* 16:107-115, 1994

36. Tonini GP, Boni L, Pession A, et al: MYCN oncogene amplification in neuroblastoma is associated with worse prognosis, except in stage 4s: The Italian experience with 295 children. *J Clin Oncol* 15:85-93, 1997

37. Maris JM, Matthay KK: Molecular biology of neuroblastoma. *J Clin Oncol* 17:2264-2279, 1999