*Journal of Pediatric Hematology/Oncology 21(3): 181–189, May/June 1999*


# Metastatic Sites in Stage IV and IVS Neuroblastoma Correlate With Age, Tumor Biology, and Survival

Steven G. DuBois, M.D., Yan Kalika, D.D.S., John N. Lukens, M.D., Garrett M. Brodeur, M.D., Robert C. Seeger, M.D., James B. Atkinson, M.D., Gerald M. Haase, M.D., C. Thomas Black, M.D., Carlos Perez, M.D., Hiroyuki Shimada, M.D., Robert Gerbing, M.A., Daniel O. Stram, Ph.D., and Katherine K. Matthay, M.D.

**Purpose:** The goal of this study was to determine the incidence of metastatic sites in neuroblastoma and the extent to which metastatic sites correlate with age, tumor biology, and survival.
**Patients and Methods:** All 648 patients with stage IV and IVS neuroblastoma registered on Children's Cancer Group protocols 3881 and 3891 were analyzed. Metastatic site data were provided by treating institutions and reviewed in patients with central nervous system (CNS), intracranial, lung, or "other" metastases.
**Results:** The incidence of metastatic sites at diagnosis was 70.5% in bone marrow, 55.7% in bone, 30.9% in lymph nodes, 29.6% in liver, 18.2% in intracranial and orbital sites, 3.3% in lung, and 0.6% in CNS. Event-free survival (EFS) was decreased in patients with bone, bone marrow, CNS, intracranial/orbital, lung, and pleural metastases, and improved in those with liver and skin metastases. In infants, *MYCN* amplification and unfavorable Shimada histopathology correlated with increased frequencies of bone and intracranial or orbital metastases. In older patients, *MYCN* amplification correlated with increased frequencies of intracranial or orbital, liver, and lung metastases. Multivariate analysis revealed that metastatic site is not an independent prognostic factor.
**Conclusions:** Metastatic pattern in neuroblastoma differs with age and correlates with tumor biological features and EFS. These correlations could reflect changes in host or tumor biological features with age resulting in differences in metastatic capacity or tumor affinity for specific sites.
**Key Words:** Neuroblastoma—Metastasis—Age—*MYCN*—Survival.

The majority of children with neuroblastoma have metastatic disease at diagnosis, yet even this group is heterogeneous with respect to outcome. Many clinical and biological factors, including age and tumor biology, have been shown to affect prognosis (1,2). In certain instances, the specific sites of metastasis may be prognostic. Perhaps the best characterized example of metastatic sites influencing survival is the favorable subset of infants (Evans stage IVS) with metastases restricted to the bone marrow, skin, or liver but without bony metastases (3). Other authors have attempted to determine the prognostic value of specific metastatic sites, including bone, bone marrow, skin, and distant lymph nodes (4–10). The conclusions in many of these studies are equivocal due to small sample sizes, and many of these reports are based on studies that examine only one metastatic site, making it difficult to compare the effect of each site on survival between studies.

In addition to metastatic site, several biological features of neuroblastoma tumors have been identified that have prognostic value, including serum ferritin levels, *MYCN* oncogene copy number, and Shimada histopathology classification (11–13). Although their effect on outcome has been studied, the relationship between these

Submitted for publication May 22, 1998; accepted October 5, 1998.
From the Department of Pediatrics, University of California School of Medicine (S.G.D., Y.K., K.K.M.), San Francisco, California; Department of Pediatrics, Vanderbilt University School of Medicine (J.N.L.), Nashville, Tennessee; Department of Pediatrics (G.M.B.), University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; Departments of Pediatrics (R.C.S.) and Pathology (H.S.), University of Southern California School of Medicine and Children's Hospital, Los Angeles, California; Department of Surgery, Childrens Hospital (G.M.H.), Denver, Colorado; Department of Surgery, M. D. Anderson Hospital (C.T.B.), Houston, Texas; Department of Surgery, University of California School of Medicine (J.B.A., C.P.), Los Angeles, California; and Children's Cancer Group (R.G., D.O.S.), Arcadia, California, U.S.A.
Contributing Children's Cancer Group Investigators, Institutions, and Grant Numbers are given in the Appendix. Grant support CA 13539 (Childrens Cancer Group), CA 22794, CA 60104, CA 02649 (RCS), and CA 39771 (GMB) from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, and Department of Health and Human Services.
Address correspondence and reprint requests to Dr. Katherine K. Matthay, Children's Cancer Group, P.O. Box 60012, Arcadia, CA 91066-6012.

biological features and metastatic pattern in neuroblastoma has not been fully examined.

In this report, a group of 648 patients with metastatic neuroblastoma, treated on two concurrent protocols, with analyses of the extent to which metastatic site correlates with age, tumor biology, and survival is described. By combining all infants into one group for many of the analyses, the results presented provide an understanding of metastatic disease independent of any external classifications.

## PATIENTS AND METHODS

### Patients

All patients with Evans stage IV and IVS neuroblastoma registered on Children's Cancer Group (CCG) protocols 3881 (open June 1989 to August 1995) or 3891 (open January 1991 to April 1996) were included in this study. All patients with stage IV and IVS tumors registered on CCG 3881 were younger than 1 year at diagnosis (the median ages of 81 patients with stage IVS tumor and 115 patients with stage IV tumors were 0.2 years and 0.6 years, respectively). All stage IV patients registered on CCG 3891 (n = 434) were between 1 and 18 years old at diagnosis (median age, 3.0 years) or were younger than 1 year at diagnosis and had tumor *MYCN* amplification (n = 18; median age, 0.7 years). Median follow-up time was 44.1 months for patients with stage IVS tumors, 47.3 months for patients with stage IV tumors younger than 1 year, and 24.8 months for patients with stage IV tumors age 1 year or older. The cut-off date for analyses was February 1, 1997.

Stage IV patients treated on CCG 3881 received approximately 9 months of chemotherapy consisting of cisplatin, cyclophosphamide, doxorubicin, and etoposide (14). Stage IVS patients registered on CCG 3881 received only supportive care, except those patients in whom tumor growth threatened survival by compromising respiratory, renal, or hepatic function (n = 37). These patients received 1 or 2 courses of low-dose (5 mg/kg/d for 7 days) cyclophosphamide, usually with limited hepatic radiation. Patients with stage IV tumor treated on CCG 3891 received approximately 9 months of more intensive chemotherapy with the same 4 agents used in CCG 3881 followed by a consolidation course of either myeloablative therapy with autologous bone marrow transplantation or chemotherapy with cisplatin, etoposide, doxorubicin, and ifosfamide (14).

### Sites of Disease

Treating institutions completed standardized CCG 3881 and 3891 protocol forms at the time of treatment indicating sites of primary and metastatic disease at diagnosis and progression. Primary tumor sites and overall staging were centrally reviewed for all patients by a minimum of two CCG surgeons and the study chair. CCG 3881 and 3891 protocols required studies to assess metastatic spread, including bone marrow aspirate (completed for 99% of all patients at diagnosis), biopsy (93%), bone scan (97%), computed tomography (CT) or ultrasound (97%), chest X-ray (92%), and skeletal survey (66%). MIBG scan (18%) was recommended but not required because of lack of availability at all institutions.

In patients in whom central nervous system (CNS), intracranial, lung, or "other" were indicated as a metastatic site, the patients' records, including standardized forms and radiology, pathology, and surgery reports, were reviewed by the authors (S.G.D., Y.K., and K.K.M.) to confirm the site. Corrections were approved by the treating institutions. CNS metastasis was defined as tumor in the cerebrospinal fluid, leptomeninges, or parenchyma of the brain or spinal cord, excluding paraspinal disease. Intracranial metastasis was defined as tumor arising from the dura, epidural space, or bones of the skull. Lung metastasis was defined as tumor in the lung parenchyma. For the rare patients for whom information confirming the site was not available because of the multi-institutional nature of this study, the classification of the treating institution was retained. Records of patients age 1 year or older at diagnosis reported to have had metastases restricted to distant lymph nodes or other anatomic sites likely to represent lymph node involvement (thorax, pelvis, neck, other abdominal, and thoracoabdominal) at diagnosis were also reviewed to confirm that metastases were restricted to distant lymph nodes. Other patients reported to have had lymph node metastases may have had distant or regional node involvement.

Seventeen occurrences of CNS metastases and 36 occurrences of lung metastases were confirmed by CT (n = 34); pathology (n = 6); pathology and CT, magnetic resonance imaging (MRI), or X-ray (n = 6); MRI (n = 2); X-ray (n = 2); clinical summary note (n = 2); and CT and MRI (n = 1). Six reports of lung metastases could not be confirmed from available information. The classifications of CNS, lung, or intracranial were changed in 38 of 144 cases reviewed in which patients were reported to have metastases to these sites. The majority of these changes resulted from inconsistencies in the distinction between CNS and intracranial metastases. Thus, this proportion of changes (26%) does not necessarily reflect a similar error rate in the reporting of sites encountered more frequently with this disease. Sites coded as "other" were reassigned to appropriate preexisting categories for 81 of 94 patients. Eleven patients with stage IV tumor age 1 year or older with metastases restricted to distant lymph nodes were confirmed by review of a combination of pathology reports and standardized surgical forms from diagnosis.

### Biological Characteristics

Tumor histopathology was reviewed centrally according to the Shimada histopathology classification (13). *MYCN* copy number was determined in the CCG Reference Laboratory either by Southern analysis (before 1993) (15) or by assaying MYCN protein expression with semiquantitative immunoperoxidase staining (after 1993) (16). Tumors with ≥10 copies of *MYCN* at diagnosis were classified as *MYCN* amplified, and tumors with <10 copies were classified as unamplified. Only central determinations of Shimada histopathology classification and *MYCN* copy number were included in this study. Tumor tissue was not available at diagnosis for all patients because, in some patients, the diagnosis of neuroblastoma was made based on elevated urine catecholamines with typical tumor cells in the bone marrow. Serum ferritin levels at diagnosis were determined by radioimmunoassay performed by the treating institution. Serum ferritin level of ≥143 ng/mL was designated as elevated, and serum ferritin <143 ng/mL was designated as normal (11).

### Statistical Analysis

Life table analyses were used to estimate the event-free survival (EFS) from the date of diagnosis. Comparison of EFS probabilities between subgroups was made using the log-rank statistic (17). Multivariate analysis using the regression method of Cox was performed. The independent prognostic power of factors, including metastatic sites, *MYCN* amplification, Shimada histopathology, and age, were considered by fitting a Cox model for the joint effect on the risk for an adverse event of these factors simultaneously. A factor was judged to provide significant prognostic information if it added significantly ($P < 0.05$) to the fit of the multivariate model. Chi-square analysis was used for other comparisons.

## RESULTS

### Sites of Primary and Metastatic Disease

For the 648 patients included in this study, the adrenal gland was the most common site of primary disease (69%). Almost all of the remaining patients had primary disease arising along the sympathetic chain. There were two patients in whom the reported primary site was the head/orbit and one patient in whom the reported primary site was within the subdural space of the spinal cord. In these three patients, the true primary tumor probably was unidentifiable. Patients with stage IVS tumor were less likely to have nonadrenal abdominal primary tumors than patients with stage IV tumor ($P < 0.01$; data not shown). Otherwise, the distribution of primary tumor sites did not differ significantly between subgroups.

Table 1 shows the important differences in metastatic pattern between age and stage groups at diagnosis. Infants with stage IV or IVS disease had a higher frequency of skin and liver metastases and a lower frequency of bone and bone marrow metastases. Infants with stage IVS tumor were more likely to have liver metastases than infants with stage IV tumor. Adrenal metastases were reported in 49 patients at diagnosis; 44 of these patients had adrenal primary tumors. Although the most likely explanation for these 44 occurrences of adrenal metastases is that they represent bilateral adrenal involvement,

**TABLE 1.** *Sites of metastasis at diagnosis for 81 patients with stage IVS, 133 patients with stage IV <1 year, and 434 patients with stage IV ≥1 year*

| | Stage IVS n (%) | Stage IV <1 year, n (%) | Stage IV ≥1 year, n (%) | Total % |
|---|---|---|---|---|
| Bone marrow*,† | 28 (34.6) | 76 (57.1) | 353 (81.3) | 70.5 |
| Bone† | 0 (0.0) | 65 (48.9) | 296 (68.2) | 55.7 |
| Lymph node | 7 (8.6) | 38 (28.6) | 155 (35.7) | 30.9 |
| Liver*,† | 65 (80.2) | 71 (53.4) | 56 (12.9) | 29.6 |
| Intracranial/Orbit | 0 (0.0) | 34 (25.6) | 84 (19.6) | 18.2 |
| Adrenal† | 5 (6.2) | 18 (13.5) | 26 (6.0) | 7.6 |
| Skin† | 11 (13.6) | 11 (8.3) | 4 (0.9) | 4.0 |
| Pleura | 0 (0.0) | 6 (4.5) | 16 (3.7) | 3.4 |
| Lung | 0 (0.0) | 3 (2.3) | 18 (4.1) | 3.2 |
| Peritoneum | 0 (0.0) | 5 (3.8) | 9 (2.1) | 2.2 |
| Other | 0 (0.0) | 5 (3.8) | 7 (1.6) | 1.9 |
| Central nervous system | 0 (0.0) | 0 (0.0) | 4 (0.9) | 0.6 |

*Significant difference between the proportion of patients with stage IVS with the site and the proportion of patients with stage IV <1 year with the site ($P < 0.01$), only for those sites which are included in the definition of stage IVS.

†Significant difference between the proportion of patients with stage IV <1 year with the site and the proportion of patients with stage IV ≥1 year with the site ($P < 0.02$).

this could not be confirmed from available data. Other unusual sites reported in patients with stage IV tumor younger than 1 year included paratesticular (n = 2), testicular (n = 1), pituitary (n = 1), and, in 1 patient, the site referred to as "other" could not be determined from available information. Other sites in patients with stage IV age 1 year or older included maxillary sinus (n = 4), parotid gland (n = 2), and lacrimal duct (n = 1). A group of patients were reported to have metastases categorized by anatomic site, including abdominal, other (9.0% of all patients at diagnosis), thorax (6.6%), pelvis (4.8%), neck (3.1%), and thoracoabdominal (3.1%). It is likely that the majority of these metastases reflect involvement of lymphatic tissue. Because the exact nature of these metastases could not be determined, they have not been included in any analyses.

Table 2 summarizes the sites of metastases at first progression in 334 patients, including 10% of patients with stage IVS tumor, 34% of patients with stage IV tumor younger than 1 year, and 65% of patients with stage IV tumor age 1 year or older with progressive disease. The only site referred to as "other" at first progression was a testicular metastasis in a patient with stage IV tumor age 1 year or older. Patients with stage IV tumor age 1 year or older were more likely to have bone and bone marrow metastases and less likely to have liver, intracranial or orbital, and CNS metastases at first progression than patients with stage IV tumor younger than 1 year. The overall distribution of metastases at first progression differed from the distribution of metastases at diagnosis. The frequencies of bone, bone marrow, liver, lymph node, and skin metastases were lower at first progression than at diagnosis, and the frequencies of adrenal, lung, and CNS metastases were higher at first progression than at diagnosis.

Table 3 summarizes the specific features of the patients in this study with confirmed CNS involvement at diagnosis or new CNS involvement at first progression. The 4 patients with CNS metastases at diagnosis were older than 1 year at diagnosis, had CNS involvement restricted to the leptomeninges, and died within 1 year of diagnosis. These patients all had primary tumors or intracranial metastases that could reasonably be expected to have infiltrated the cerebrospinal fluid spaces or leptomeninges. Therefore, at diagnosis, hematogenous spread resulting in discrete parenchymal metastases was not observed. Similar to the pattern seen at diagnosis, 4 of the 5 patients younger than 1 year with CNS metastases at progression had only leptomeningeal involvement. The pattern of CNS metastases at progression in patients age 1 year or older was different from that observed at diagnosis or in infants at progression. These older children with new CNS metastases at progression all had parenchymal involvement that largely occurred in the absence of previous or concurrent intracranial metastases, implying hematogenous spread rather than direct extension of tumor. All patients with CNS metastases who were tested had tumor *MYCN* amplification and unfavorable Shimada histopathology.

The specific features of patients with lung metastases at diagnosis or new lung involvement at first progression are shown in Table 4. *MYCN* amplification was found in all but one patient at diagnosis, but in only two patients with new involvement at progression. Outcome was poor in patients with lung involvement at diagnosis or progression, and most of these patients died of disease.

**TABLE 2.** *Sites of metastasis at first progression in the 8 patients with stage IVS, the 45 patients with stage IV <1 year, and the 281 patients with stage IV ≥1 year with progressive disease*

| | Stage IVS n (%) | Stage IV <1 year, n (%) | Stage IV ≥1 year, n (%) | Total % |
|---|---|---|---|---|
| Bone*† | 1 (12.5) | 15 (33.3) | 142 (50.5) | 47.3 |
| Bone marrow*† | 2 (25.0) | 7 (15.6) | 139 (49.5) | 44.3 |
| Intracranial/orbit* | 0 (0.0) | 16 (35.6) | 45 (16.0) | 18.3 |
| Adrenal† | 4 (50.0) | 10 (22.2) | 46 (16.4) | 18.0 |
| Liver*† | 5 (62.5) | 12 (26.7) | 35 (12.5) | 15.6 |
| Lymph node† | 3 (37.5) | 4 (8.9) | 33 (11.7) | 12.0 |
| Lung† | 1 (12.5) | 2 (4.4) | 18 (6.4) | 6.3 |
| Central nervous system*† | 0 (0.0) | 5 (11.1) | 8 (2.8) | 3.9 |
| Peritoneum | 0 (0.0) | 2 (4.4) | 6 (2.1) | 2.4 |
| Pleura | 0 (0.0) | 1 (2.2) | 7 (2.5) | 2.4 |
| Skin† | 0 (0.0) | 1 (2.2) | 2 (0.7) | 0.9 |
| Testicle | 0 (0.0) | 0 (0.0) | 1 (0.4) | 0.3 |

*Significant difference between the proportion of patients with stage IV <1 year with the site and the proportion of patients with stage IV ≥1 year with the site ($P < 0.05$).

†Significant difference between the proportion of all patients with the site at diagnosis and the proportion of all patients with the site at first progression ($P < 0.03$).

**TABLE 3.** *Features of patients with confirmed central nervous system metastasis at diagnosis or new involvement at first progression*

| | | Progression | |
|---|---|---|---|
| | Diagnosis (n = 4) | Stage IV <1 year (n = 5) | Stage IV ≥1 year (n = 7) |
| Median age and range (y) | 1.6 (1.1–10.2) | 0.5 (0.4–0.7) | 2.1 (1.7–4.0) |
| Brain or cord parenchyma involved | 0 | 1 | 7 |
| Leptomeninges involved | 4 | 4 | 2 |
| Other intracranial involvement | 3 | 2 | 2 |
| Died of disease | 4 | 5 | 7 |
| Ferritin elevated/total tested | 2/3 | 1/5 | 3/4 |
| *MYCN* amplified/total tested | 1/1 | 3/3 | 4/4 |
| Shimada unfavorable/total tested | 2/2 | 1/1 | 3/3 |

Intracranial involvement indicates whether the patient had previous or concurrent intracranial metastases. The total number of patients does not equal 17, as shown in Tables 1 and 2, because one patient with central nervous system disease at progression also had central nervous system disease at diagnosis.

## Correlation of Metastatic Sites With Event-free Survival

Event-free survival was significantly higher for infants (stage IVS and IV tumor younger than 1 year combined; 3-year EFS, 72%) than for older children (stage IV tumor age 1 year or older; 3-year EFS, 23%; $P < 0.001$). EFS for all patients was compared according to the presence or absence of a specific metastatic site at diagnosis (Table 5 and Fig. 1). Bone, bone marrow, CNS, intracranial or orbital, lung, and pleura were all significant unfavorable prognostic sites, although liver and skin were significant favorable prognostic sites. When adjusted for age, only bone, intracranial or orbital, and lung sites were significantly unfavorable for infants and older children. Within the group of patients without *MYCN* amplification, bone metastases were significantly unfavorable (3-year EFS for patients with bone metastases, 38%; 3-year EFS for patients without bone metastases, 70%; $P < 0.001$). Skin metastases were favorable and bone marrow and pleural metastases were unfavorable in infants. CNS and liver metastases were unfavorable in older children. The study population included 11 patients with stage IV tumor age 1 year or older with only distant lymph node metastases at diagnosis. Although there was a trend indicating that stage IV patients age 1 year or older with only distant lymph node metastases had a higher EFS than the remaining stage IV patients age 1 year or older, this difference was not significant (Fig. 1D).

**TABLE 4.** *Features of patients with lung metastasis at diagnosis or new involvement at first progression*

| | Diagnosis (n = 21) | Progression (n = 16) |
|---|---|---|
| Median age and range (y) | 2.1 (0.1–6.3) | 2.5 (0.1–12.6) |
| NED/AWD/DOD | 3/1/17 | 0/1/15 |
| Ferritin increased/total tested | 8/20 | 12/14 |
| *MYCN* amplified/total tested | 10/11 | 2/9 |
| Shimada unfavorable/total tested | 10/12 | 7/7 |

AWD, patients alive with disease; DOD, patients who died of disease; NED, patients alive with no evidence of disease.

The total number of patients does not equal 42, as shown in Tables 1 and 2, because five patients with lung disease at progression also had lung disease at diagnosis.

## Correlation of Metastatic SitesWith Biological Features

Serum ferritin was elevated in 24 of 53 (45.3%) patients tested with stage IVS tumor, 38 of 114 (33.3%) patients tested with stage IV tumor younger than 1 year, and 234 of 384 (60.9%) patients tested with stage IV age 1 year or older. *MYCN* oncogene was amplified in no patients tested with stage IVS tumor, 31 of 101 (30.7%) patients tested with stage IV tumor younger than 1 year, and 108 of 291 (37.1%) patients tested with stage IV tumor age 1 year or older. Shimada histopathology was unfavorable in 3 of 72 (4.2%) patients tested with stage IVS, 28 of 90 (31.1%) patients tested with stage IV tumor younger than 1 year, and 262 of 276 (94.9%) patients tested with stage IV tumor age 1 year or older. To determine whether specific biological features correlated with specific metastatic sites, the odds ratios for having each metastatic site at diagnosis in relation to having elevated serum ferritin, *MYCN* oncogene amplification, or unfavorable Shimada classification were calculated (Table 6). Patients age 1 year or older with *MYCN* amplification were more likely to have intracranial or orbital, liver, or lung metastases at diagnosis. Patients younger than 1 year with *MYCN* amplification or unfavorable Shimada histopathology were also at increased risk for intracranial or orbital metastases and bone metastases. Infants with *MYCN* amplification or unfavor-

**TABLE 5.** *Comparison of 3 year percentage event-free survival for patients with (+) and without (–) specific metastatic sites at diagnosis*

| | All patients | | Stages IVS and IV <1 year | | Stage IV ≥1 year | |
|---|---|---|---|---|---|---|
| | + | – | + | – | + | – |
| Adrenal | 43 | 41 | 68 | 72 | 20 | 23 |
| Bone | 26 | 58* | 55 | 79* | 18 | 33* |
| Bone marrow | 33 | 58* | 63 | 79* | 23 | 23 |
| Central nervous system | 0 | 41* | — | — | 0 | 23* |
| Intracranial/orbit | 25 | 44* | 45 | 77* | 18 | 24* |
| Liver | 58 | 33* | 73 | 69 | 20 | 23* |
| Lung | 14 | 42* | 0 | 73* | 19 | 23* |
| Lymph node | 35 | 44 | 69 | 72 | 21 | 24 |
| Peritoneum | 45 | 41 | 60 | 72 | 32 | 23 |
| Pleura | 21 | 42* | 50 | 72* | 15 | 23 |
| Skin | 80 | 39* | 96 | 69* | 0 | 23 |

*Significant log rank difference ($P < 0.05$) between patients with and without the site.

able Shimada histopathology had a significantly lower likelihood of having liver metastases at diagnosis.

### Multivariate Analysis of Event-free Survival in Relation to Metastatic Site, Tumor Biology, and Age

To determine whether certain metastatic sites predicted EFS independent of *MYCN* amplification, Shimada histopathology, or age, a multivariate analysis was performed. Bone, bone marrow, and intracranial or orbital metastatic sites were included because these sites correlated with EFS and tumor biology in univariate analyses and were observed in adequate numbers to allow for analysis. Serum ferritin was not included in the analysis because it correlated only weakly with bone marrow metastases. The results confirm the well-known independent prognostic value of *MYCN* amplification, Shimada histopathology, and age ($P < 0.001$). However, bone, bone marrow, and intracranial or orbital metastatic sites were not found to be independent prognostic factors.

## DISCUSSION

The results indicate that metastatic pattern differs with age. Patients with stage IV tumor younger than 1 year were more likely to have liver or skin metastases at diagnosis and less likely to have bone and bone marrow metastases at diagnosis than patients with stage IV tumor age 1 year or older. This pattern parallels the well-characterized stage IVS designation and may indicate that all infants with stage IV and IVS tumor share important features leading to similar metastatic tendencies. Patients with stage IV tumor younger than 1 year were more likely to have bone marrow involvement and less likely to have liver involvement than patients with stage IVS. Understanding the bases for these similarities and differences may provide insight into factors underlying the difference in survival between these groups.

The large number of patients in this study provided the opportunity to consider additional features of two unusual sites of metastasis: lung and CNS. The overall frequencies of lung involvement (42 of 648) and CNS involvement (17 of 648) at diagnosis and at first progression were much lower than previously reported in patients at all stages at any point in their illness (18–21). Patients were also more likely to have lung or CNS involvement at first progression than at diagnosis. These findings suggest a tendency for lung and CNS metastasis to occur late in the disease; therefore, the incidence of cases only from diagnosis and first progression would be less than reports based on cases at any point in the course of disease. The implication that lung and CNS metastases may be more likely to occur later in the disease is consistent with the proposal that improvements in survival time increase the opportunity for spread to unusual sites, possibly because of the emergence of unusually aggressive clones of cells (20,21). Lung and CNS metastases at diagnosis were associated with a very poor outcome, statistically confirming the trend observed in previous reports (18,19,21–23).

The nature of CNS and lung metastases may be different at diagnosis than at relapse. CNS involvement at diagnosis appeared to result from direct extension of tumor, although the greater degree of discrete parenchymal involvement at progression was suggestive of hematogenous spread. Although some authors have reported simultaneous occurrences of hematogenous metastases to the CNS and lung (22,24), only one of the patients in this study followed this pattern. The mechanism of lung metastasis at progression may also differ from diagnosis because *MYCN* amplification is commonly seen in patients with lung metastases at diagnosis but only rarely at progression.

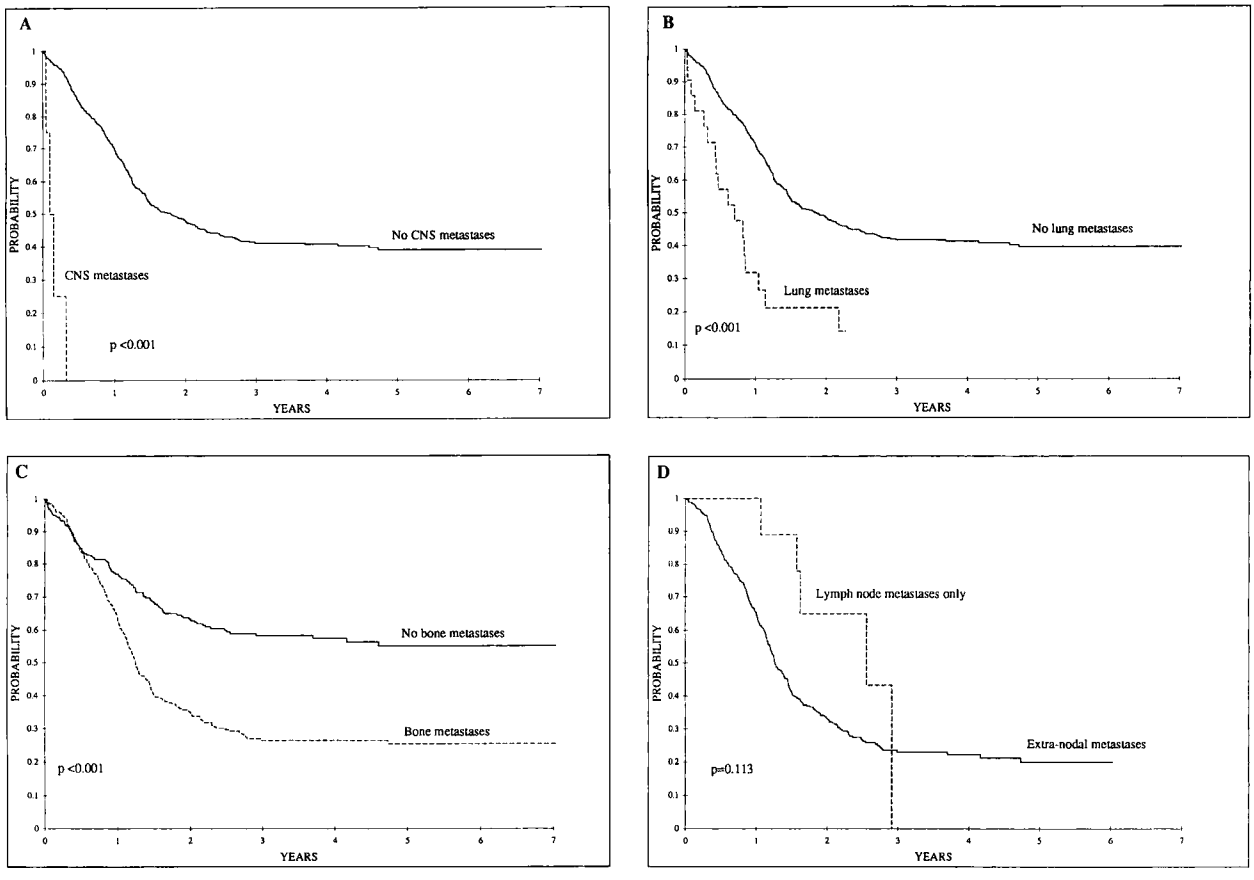


**FIG. 1.** Estimated event-free survival curves from diagnosis for all patients with and without metastases to the central nervous system **(A)**, lung **(B)**, and bone **(C)**. **D:** Patients with stage IV disease age 1 year or older with metastases only to lymph nodes compared to patients with stage IV disease age 1 year or older with extranodal metastases.

A number of factors potentially limit the accuracy of the metastatic site data. The large number of patients and the multi-institutional nature of this study made a review of the metastatic evaluation for all patients impossible. The metastatic sites provided by the treating institutions were assumed to be correct, except for less commonly encountered sites, such as lung, CNS, or distant lymph node-only sites. Because errors in assessing these rare sites would have a greater impact on the results, a central review of pathology and radiology reports of these patients was performed. Because most of these studies were from children's hospitals or major university centers, this type of review was probably sufficient. For more common sites, the large number of patients diminishes the impact of a few possible errors. The accuracy of the reported frequencies of lymph node metastases at diagnosis is difficult to assess because regional nodes were not assessed by surgery at diagnosis for all patients. Because all patients were not routinely evaluated by CT scan for metastasis to less commonly observed sites, particularly CNS, which may have been asymptomatic, the reported frequencies may underestimate the true incidence. The frequency of CNS metastases could be determined more accurately with a prospective study in which all patients receive head CT or MRI scans, but the low incidence of CNS metastases does not justify routine scans in asymptomatic patients.

The results indicate that certain metastatic sites correlate with EFS. With the possible exception of CNS metastasis, it seems unlikely that the presence of metastatic disease in certain sites actually causes the decreased EFS. Instead, the observed correlations between EFS and metastatic sites probably reflect a tendency for metastasis to these sites to occur more frequently in patients with more aggressive disease. This idea is supported by the close correlations between metastatic sites and tumor biology and by the finding that metastatic site is not independent of tumor biology in determining EFS. Thus, although metastatic site may be useful in determining prognosis in patients in whom tumor biological features have not been evaluated, the multivariate analysis highlights the overriding importance of tumor biology as a prognostic factor.

The correlations between metastatic site and tumor

**TABLE 6.** *Relationship between site of metastasis at diagnosis and tumor biological features*

| | Stages IVS and IV <1 year | | | Stage IV ≥1 year | | |
|---|---|---|---|---|---|---|
| | FE | *MYCN* | SHIM | FE | *MYCN* | SHIM |
| Adrenal | 0.857 | 1.76 | 1.33 | 1.30 | 1.22 | 1.06 |
| Bone | 0.870 | 2.41* | 2.05* | 1.05 | 1.01 | 1.01 |
| Bone marrow | 0.974 | 1.39 | 1.52* | 1.06* | 0.952 | 1.00 |
| Central nervous system | — | — | — | 1.11 | 3.33 | 1.05 |
| Intracranial/orbit | 0.625 | 2.95* | 2.63* | 1.07 | 1.35* | 1.03 |
| Liver | 1.14 | 0.681* | 0.615* | 1.13 | 1.56* | 1.00 |
| Lung | 0.909 | 0.000 | 0.000 | 0.680 | 2.70* | 1.06 |
| Lymph node | 0.839 | 1.45 | 2.06* | 1.04 | 0.907 | 0.974 |
| Peritoneum | 1.110 | 2.22 | 2.00 | 1.43 | 1.15 | 1.05 |
| Pleura | 0.556 | 2.22 | 0.000 | 0.960 | 1.08 | 1.06 |
| Skin | 0.847 | 0.000 | 0.000 | 1.25 | 1.82 | 1.07 |

FE, increased serum ferritin levels; *MYCN*, *MYCN* amplification; SHIM, unfavorable Shimada histology classification.

*Significant odds ratio for having the specific site if the patient is positive for the given biological feature ($P < 0.05$).

The table entry represents the odds ratio (observed/expected) for having the specific site if the patient is positive for the given biological feature.

biology raise the possibility that specific biological features may be required for metastasis to specific sites. Recent reports suggest that specific factors may be involved in targeting the spread of neuroblastoma to the liver (25,26). In this study, the finding that liver metastases were less likely in infants with *MYCN* amplification but more likely in older patients with *MYCN* amplification indicates that tumor biology alone does not sufficiently account for all metastatic tendencies and highlights the importance of age in determining metastasis to this site. Although almost all children with lung metastases at diagnosis had tumor *MYCN* amplification, most patients with tumor *MYCN* amplification did not have lung metastases. It seems likely that other tumor or host characteristics, such as CD44, integrins, and nm23 expression, influence metastatic preference (27–29).

This study provides data on the incidence of metastatic sites at diagnosis and first progression in a large group of uniformly evaluated patients treated according to the protocol guidelines of two concurrent studies. The results demonstrate that certain metastatic sites correlate with age, tumor biology, and survival, but indicate that metastatic site is not an independent prognostic factor. An improved understanding of the factors influencing metastasis could lead to new insights into the requirements for tumor growth and direct the development of new chemotherapeutic agents.

## REFERENCES


1. Brodeur GM, Castlebery RP. Neuroblastoma. In: Pizzo PA, Poplack DG, eds. *Principles and practice of pediatric oncology*, 3rd ed. Philadelphia: Lippincott, 1997:761–97.
2. Matthay KK. Neuroblastoma. In: Pochedly C, ed. *Neoplastic diseases in childhood*. Chur, Switzerland: Harwood, 1994:735–78.
3. D'Angio GJ, Evans AE, Koop CE. Special pattern of widespread neuroblastoma with a favourable prognosis. *Lancet* 1971;1: 1046–9.
4. Daubenton JD, Fisher RM, Karabus CD, et al. The relationship between prognosis and scintigraphic evidence of bone metastases in neuroblastoma. *Cancer* 1987;59:1586–9.
5. Labreveux de Cervens C, Hartmann O, Bonnin F, et al. What is the prognostic value of osteomedullary uptake on MIBG scan in neuroblastoma patients under one year of age? *Med Pediatr Oncol* 1994;22:107–14.
6. Nakagawara A, Morita K, Okabe I, et al. Proposal and assessment of Japanese Tumor Node Metastasis postsurgical histopathological staging system for neuroblastoma based on an analysis of 495 cases. *Jpn J Clin Oncol* 1991;21:1–7.
7. Moss TJ, Reynolds CP, Sather HN, et al. Prognostic value of immunocytologic detection of bone marrow metastases in neuroblastoma. *N Engl J Med* 1991;324:219–26.
8. Stephenson SR, Cook BA, Mease AD, et al. The prognostic significance of age and pattern of metastases in stage IV-S neuroblastoma. *Cancer* 1986;58:372–5.
9. Berthold F, Trechow R, Utsch S, et al. Prognostic factors in metastatic neuroblastoma. *Am J Pediatr Hematol Oncol* 1992;14:207–15.
10. Rosen EM, Cassady R, Frantz CN, et al. Stage IV-N: a favorable subset of children with metastatic neuroblastoma. *Med Pediatr Oncol* 1985;13:194–8.
11. Hann HWL, Evans AE, Siegel SE, et al. Prognostic importance of serum ferritin in patients with stages III and IV neuroblastoma: the Children's Cancer Study Group experience. *Cancer Res* 1985;45: 2843–8.
12. 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* 1985;313:1111–6.
13. Shimada H, Chatten J, Newton WA, 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.
14. Matthay KK, Perez C, Seeger RC, et al. Successful treatment for stage III neuroblastoma based on prospective biologic staging: a Children's Cancer Group Study. *J Clin Oncol* 1998;16:1256–64.
15. Brodeur GM, Seeger RC, Schwab M, et al. Amplification of N-

myc in untreated human neuroblastomas correlates with advanced disease stage. *Science* 1984;224:1121–4.
16. 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* 1988;271:41–9.
17. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457–81.
18. Cowie F, Corbett R, Pinkerton CR. Lung involvement in neuroblastoma: incidence and characteristics. *Med Pediatr Oncol* 1997; 28:429–32.
19. Towbin R, Gruppo RA. Pulmonary metastases in neuroblastoma. *Am J Roentgenol* 1982;138:75–8.
20. Carlsen NLT, Schroeder H, Christensen IJ, et al. Signs, symptoms, metastatic spread and metabolic behavior of neuroblastomas treated in Denmark during the period 1943–1980. *Anticancer Res* 1987;7:465–74.
21. Blatt J, Fitz C, Mirro JR. Recognition of central nervous system metastases in children with metastatic primary extracranial neuroblastoma. *Pediatr Hematol Oncol* 1997;14:233–41.
22. Kellie SJ, Hayes FA, Bowman L, et al. Primary extracranial neuroblastoma with central nervous system metastases: characterization by clinicopathologic findings and neuroimaging. *Cancer* 1991;68:1999–2006.
23. Shaw PJ, Eden T. Neuroblastoma with intracranial involvement: an ENSG Study. *Med Pediatr Oncol* 1992;20:149–55.
24. Vannucci RC, Baten M. Cerebral metastatic disease in childhood. *Neurology* 1974;24:981–5.
25. Tsuchida Y, Yokomori K, Saito S, et al. Stage IV-S neuroblastoma involving the liver and ectopic liver. *Cancer* 1984;53:1609–11.
26. Kuwashima N. Organ-specific adhesion of neuroblastoma cells in vitro: correlation with their hepatic metastasis potential. *J Pediatr Surg* 1997;32:546–51.
27. Gross N, Beretta C, Peruisseau G, et al. CD44H expression by human neuroblastoma cells: relation to *MYCN* amplification and lineage differentiation. *Cancer Res* 1994;54:4238–42.
28. Favrot MC, Combaret V, Goillot E, et al. Expression of integrin receptors on 45 clinical neuroblastoma specimens. *Int J Cancer* 1991;49:347–55.
29. Leone A, Seeger RC, Hong CM, et al. Evidence for *nm23* RNA overexpression, DNA amplification and mutation in aggressive childhood neuroblastomas. *Oncogene* 1993;8:855–65.