Title

Clinical, Biologic, and Prognostic Differences on the Basis of Primary Tumor Site in Neuroblastoma: A Report From the International Neuroblastoma Risk Group Project

Journal

Journal of Clinical Oncology, 32(28)

ISSN

0732-183X

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Publication Date

2014-10-01

DOI

10.1200/jco.2014.56.1621

Peer reviewed

VOLUME 32 · NUMBER 28 · OCTOBER 1 2014

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

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Published online ahead of print at www.jco.org on August 25, 2014.

Support information appears at the end of this article.

Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

Data included in the International Neuroblastoma Risk Group database were provided by the Children's Oncology Group (COG), Pediatric Oncology Group (POG), Children's Cancer Study Group (CCSG), German Gesellschaft für Pädiatrische Onkologie und Hämatologie (GPOH), European Neuroblastoma Study Group (ENSG), International Society of Paediatric Oncology Europe Neuroblastoma Group (SIOPEN), Japanese Advanced Neuroblastoma Study Group (JANB), Japanese Infantile Neuroblastoma Co-operative Study Group (JINCS), Spanish Neuroblastoma Group, and Italian Neuroblastoma Group. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the funding sources listed.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/14/3228w-3169w/$20.00

DOI: 10.1200/JCO.2014.56.1621

Clinical, Biologic, and Prognostic Differences on the Basis of Primary Tumor Site in Neuroblastoma: A Report From the International Neuroblastoma Risk Group Project

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ABSTRACT

Purpose
Neuroblastoma (NB) is a heterogeneous tumor arising from sympathetic tissues. The impact of primary tumor site in influencing the heterogeneity of NB remains unclear.

Patients and Methods
Children younger than age 21 years diagnosed with NB or ganglioneuroblastoma between 1990 and 2002 and with known primary site were identified from the International Neuroblastoma Risk Group database. Data were compared between sites with respect to clinical and biologic features, as well as event-free survival (EFS) and overall survival (OS).

Results
Among 8,369 children, 47% had adrenal tumors. All evaluated clinical and biologic variables differed statistically between primary sites. The features that were > 10% discrepant between sites were stage 4 disease, *MYCN* amplification, elevated ferritin, elevated lactate dehydrogenase, and segmental chromosomal aberrations, all of which were more frequent in adrenal versus nonadrenal tumors ($P < .001$). Adrenal tumors were more likely than nonadrenal tumors (adjusted odds ratio, 2.09; 95% CI, 1.67 to 2.63; $P < .001$) and thoracic tumors were less likely than nonthoracic tumors (adjusted odds ratio, 0.20; 95% CI, 0.11 to 0.39; $P < .001$) to have *MYCN* amplification after controlling for age, stage, and histologic grade. EFS and OS differed significantly according to the primary site ($P < .001$ for both comparisons). After controlling for age, *MYCN* status, and stage, patients with adrenal tumors had higher risk for events (hazard ratio, 1.13 compared with nonadrenal tumors; 95% CI, 1.03 to 1.23; $P = .008$), and patients with thoracic tumors had lower risk for events (HR, 0.79 compared with nonthoracic; 95% CI, 0.67 to 0.92; $P = .003$).

Conclusion
Clinical and biologic features show important differences by NB primary site, with adrenal and thoracic sites associated with inferior and superior survival, respectively. Future studies will need to investigate the biologic origin of these differences.

J Clin Oncol 32:3169-3176. © 2014 by American Society of Clinical Oncology

INTRODUCTION

One of the hallmarks of neuroblastoma (NB) is its clinical and biologic heterogeneity. The likelihood of cure is dependent on widely varying factors, including age, disease stage, tumor site, and biologic features.[1-3] The impact of the primary site of disease in influencing the heterogeneity of NB remains unclear.

Previous work has suggested that extra-abdominal NB tumors (cervical, thoracic, pelvic) may be associated with more favorable clinical and biologic characteristics and therefore a better outcome compared with NBs that originate from the abdomen.[4] In a retrospective analysis of 143 patients with NB, the frequency of stage 4 disease, tumor *MYCN* gene amplification, elevated lactate dehydrogenase (LDH), and elevated ferritin were all significantly lower in the extra-abdominal group than in the abdominal group. Not surprisingly, the probability of 5-year event-free survival (EFS) was higher in the extra-abdominal group (94%) than in the abdominal group (69%); however, a multivariable analysis was not performed in this study.[4] Studies focused on pelvic NB have shown conflicting results. One study observed that pelvic primary tumor sites were mainly associated with advanced disease.[5] Other studies reported that pelvic tumors represent a more favorable prognostic subgroup, particularly among patients with higher-stage disease.[6,7]

Previous work has suggested that thoracic NBs are a distinct subset of tumors that present at an earlier age and localized stages and have a more favorable outcome.[8,9]

These previous studies indicate that primary tumor site may account for some of the heterogeneity in clinical features, tumor biology, and clinical outcomes in NB. Given the small size and limited scope of these previous studies, a clear understanding of the impact of primary tumor site has not been possible. We therefore performed a comprehensive analysis of primary tumor site in NB. We used the largest available cohort of patients with this disease, those registered in the International Neuroblastoma Risk Group (INRG) database, to assess whether clinical features, tumor biologic features, and survival differ between primary tumor sites.

PATIENTS AND METHODS

Patients

A total of 8,800 patients younger than age 21 years with pathologically confirmed NB or ganglioneuroblastoma who were diagnosed/enrolled between 1990 and 2002 comprise the INRG database.[10] An enrollment cutoff of 2002 was chosen to allow for sufficient follow-up time. Patients provided consent and were enrolled onto one or more NB clinical or biologic trials in Germany, Japan, Italy, Spain, or the United Kingdom or onto a North American Children's Oncology Group study or the International Society of Pediatric Oncology Europe Neuroblastoma Group (SIOPEN) Localized Neuroblastoma European Study (LNESG1). Each country, cooperative group, and treating institution obtained institutional review board approval and informed patient consent for their respective studies. In addition to the date of diagnosis and follow-up data, information on 35 potential risk factors is included in the INRG database.[10]

Of the 8,800 patients, only those patients with an assigned primary tumor site were included in the analytic cohort for this report (N = 8,369). The six primary tumor site categories included adrenal, abdominal/retroperitoneal, neck, thoracic, pelvic, and other. The "other" primary tumor site category (n = 664) comprised patients who were originally assigned an "other" designation in the INRG database (n = 507) as well as those patients who were assigned more than one of the six primary tumor site categories listed (n = 157).

Statistical Analysis

Primary tumor site was the predictor variable of interest in this analysis. The adrenal gland was the most common site. Of the sites (neck, thoracic, pelvic) that may be associated with more favorable clinical and biologic characteristics and outcome, thoracic tumors comprise the largest group. Therefore, primary tumor site was analyzed using the six categories described and also as separate grouped binary variables: adrenal versus nonadrenal and thoracic versus nonthoracic.

Clinical and biologic dependent variables described in the INRG at initial diagnosis and analyzed in this study are listed in Table 1. For LDH and ferritin, median values from the entire INRG cohort (580 U/L and 96 ng/mL, respectively) were used to dichotomize patients as having elevated or not elevated levels following the convention used for previous INRG analyses.[10] The INRG

Table 1. Clinical and Biologic Characteristics of the INRG Analytic Cohort by Primary Tumor Site (N = 8,369)

			Primary Tumor Site												
	All (N = 8,369)†		Adrenal (n = 3,966)		Abdominal/ Retroperitoneal (n = 1,991)		Neck (n = 229)		Thoracic (n = 1,266)		Pelvic (n = 253)		Other‡ (n = 664)		
Characteristic*	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	P§
Mean age at diagnosis, months	26.4		26.6		27.9		19.5		24.5		24.4		27.7		< .001
Age ≥ 18 months at diagnosis	3,812 of 8,369	46	1,882	47	963	48	66	29	492	39	103	41	306	46	< .001
Tumor diagnosis of neuroblastoma, nodular‖	3,833 of 8,369	46	1,918	48	838	42	114	50	491	39	116	46	356	54	< .001
Enrollment/diagnosis before 1996	4,173 of 8,369	50	2,008	51	993	50	113	49	719	57	109	43	231	35	< .001
INSS stage 4	3,298 of 8,186	40	1,963	50	718	37	44	20	268	22	34	14	271	42	< .001
Serum ferritin ≥ 92 ng/mL	2,192 of 4,270	51	1,239	59	533	49	35	32	188	34	37	35	160	51	< .001
LDH ≥ 587 U/L	2,540 of 5,144	49	1,332	55	681	49	54	39	271	36	54	35	148	55	< .001
MYCN amplified¶	1,114 of 6,811	16	718	23	290	17	4	2	32	3	6	3	64	12	< .001
Ploidy ≤ 1 (diploid, hypodiploid)	1,044 of 3,541	29	485	33	279	30	21	22	121	25	17	17	121	28	.001
LOH at 1p	4,78 of 2,107	23	314	30	94	18	5	11	28	10	5	11	32	20	< .001
Gain of 17q	168 of 346	49	115	61	32	43	2	33	16	27	1	14	2	18	< .001
11q aberration	218 of 1,026	21	125	26	57	24	1	5	21	14	1	10	12	11	< .001
Pooled segmental chromosomal aberration															
LOH at 1p, gain of 17q and/or 11q aberration	681 of 2,141	32	416	39	156	29	6	13	53	19	7	15	43	27	< .001
Unfavorable INPC pathology classification	1,422 of 3,989	36	720	41	354	39	22	20	141	22	31	21	154	39	< .001
High MKI	378 of 3,047	12	219	15	96	14	7	8	21	5	3	3	32	11	< .001
Undifferentiated/poorly differentiated	2,726 of 3,239	84	1,346	85	619	85	78	90	332	78	75	68	276	88	< .001

Abbreviations: ANOVA, analysis of variance; INPC, International Neuroblastoma Pathology Classification; INRG, International Neuroblastoma Risk Group; INSS, International Neuroblastoma Staging System; LDH, lactate dehydrogenase; LOH, loss of heterozygosity; MKI, Mitosis Karyorrhexis Index.

*For each variable, only the percent with the adverse risk factor is shown.

†Adverse risk factor sample size over the total sample size with data available for the variable of interest.

‡The "other" primary tumor site category (n = 664) comprised patients who were originally assigned an "other" designation in the INRG database (n = 507) as well as those patients who were assigned more than one primary tumor site category among the adrenal, abdominal/retroperitoneal, neck, thoracic, pelvic, and other categories (n = 157).

§*P* value refers to a one-way ANOVA test (for continuous age variable) or χ^2 test for all other variables (age, tumor diagnosis, year of enrollment, INSS stage, serum ferritin, LDH, *MYCN* status, ploidy, LOH at 1p, gain of 17q, 11q aberration, pooled segmental chromosomal classification, INPC pathology classification, and MKI and grade of differentiation categories).

‖INPC diagnostic category[11]: neuroblastoma or ganglioneuroblastoma, nodular versus ganglioneuroblastoma, intermixed; ganglioneuroma, maturing subtype; or ganglioneuroblastoma, well differentiated.

¶The number of *MYCN*-amplified adrenal tumors in this study differs slightly from previous INRG studies because those patients who were assigned more than one primary tumor site were included in the "other" category for this study.[12]

Table 2. Clinical and Biologic Characteristics of the INRG Analytic Cohort by Adrenal Versus Nonadrenal and Thoracic Versus Nonthoracic Primary Tumor Sites (N = 8,369)

Characteristic*	Primary Tumor Site: Adrenal (n = 3,966) No.	%	Primary Tumor Site: Nonadrenal (n = 4,403) No.	%	P†	Primary Tumor Site: Thoracic (n = 1,266) No.	%	Primary Tumor Site: Nonthoracic (n = 7,103) No.	%	P†
Mean age at diagnosis, months	26.6		26.3		.59	24.5		26.8		.018
Age ≥ 18 months at diagnosis	1,882	47	1,930	44	.001	492	39	3,320	47	< .001
Neuroblastoma or ganglioneuroblastoma, nodular‡	1,918	48	1,915	43	< .001	491	39	3,342	47	< .001
Enrollment/diagnosis before 1996	2,008	51	2,165	49	.182	719	57	3,454	49	< .001
INSS stage 4	1,963	50	1,335	31	< .001	268	22	3,030	44	< .001
Serum ferritin ≥ 92 ng/mL	1,239	59	953	44	< .001	188	34	2,004	54	< .001
LDH ≥ 587 U/L	1,332	55	1,208	44	< .001	271	36	2,269	52	< .001
MYCN amplified	718	23	396	11	< .001	32	3	1,082	19	< .001
Ploidy ≤ 1 (diploid, hypodiploid)	485	33	559	27	.001	121	25	923	30	.032
LOH at 1p	314	30	164	16	< .001	28	10	450	25	< .001
Gain of 17q	115	61	53	34	< .001	16	27	152	53	< .001
11q aberration	125	26	93	17	.001	21	14	197	23	.015
Pooled segmental chromosomal aberration										
LOH at 1p, gain of 17q, and/or 11q aberration	416	39	265	25	< .001	53	19	628	34	< .001
Unfavorable INPC pathology classification	720	41	702	32	< .001	141	22	1,281	38	< .001
High MKI	219	15	159	10	< .001	21	5	357	14	< .001
Undifferentiated/poorly differentiated	1,346	85	1,380	83	.059	332	78	2,394	85	< .001

Abbreviations: INPC, International Neuroblastoma Pathology Classification; INRG, International Neuroblastoma Risk Group; INSS, International Neuroblastoma Staging System; LDH, lactate dehydrogenase; LOH, loss of heterozygosity; MKI, Mitosis Karyorrhexis Index.

*For each variable, only the percent with the adverse risk factor is shown.

†*P* value refers to a Student's *t* test (for continuous age variable) or χ^2 test for all other variables (age, tumor diagnosis, year of enrollment, INSS stage, serum ferritin, LDH, *MYCN* status, ploidy, LOH at 1p, gain of 17q, 11q aberration, pooled segmental chromosomal classification, INPC pathology classification, and MKI and grade of differentiation categories).

‡INPC diagnostic category[11]: neuroblastoma or ganglioneuroblastoma, nodular versus ganglioneuroblastoma, intermixed; ganglioneuroma, maturing subtype; or ganglioneuroblastoma, well differentiated.

database includes data on loss of heterozygosity (LOH)/aberration at 1p, gain of 17q, and 11q aberration. We evaluated each of these variables separately and also created a pooled variable reflecting the presence of segmental chromosomal aberration if at least one of these aberrations was present.[13] Clinical and biologic features were compared between groups defined by primary tumor site using χ^2 tests (for categorical variables) or *t* test or analysis of variance between groups (for continuous variables). We fit logistic regression models to describe the odds of having *MYCN* amplification according to primary tumor site after controlling for key potential confounders.

Clinical outcome variables that were available for analysis in the INRG database were EFS and overall survival (OS). EFS was defined as the time from study enrollment at diagnosis to first occurrence of relapse, progression, secondary malignancy, or death. Patients without an event were censored at the time of last patient contact. OS was defined as time from study enrollment until death, with living patients censored at the time of last contact. EFS and OS were estimated using Kaplan-Meier methods with survival distributions compared according to primary tumor site using a two-sided log-rank test.[14] Cox proportional hazards regression models were used to calculate the hazard ratio (HR) for increased risk of event or death while controlling for key potential confounders. Time-dependent covariates were used to test the proportional hazards assumption. Any variables that did not satisfy the proportional hazards assumption were removed as covariates from the model and instead used

Table 3. Logistic Regression Analysis of the Association Between Primary Tumor Site and *MYCN* Amplification Without (unadjusted OR) and With Adjustment (adjusted OR) for Age at Diagnosis, INSS Stage, and Grade of Differentiation

Primary Tumor Site	Unadjusted OR	Unadjusted 95% CI	Unadjusted *P*	Adjusted OR	Adjusted 95% CI	Adjusted *P*
Adrenal	1	Ref	Ref	1	Ref	Ref
Abdominal/retroperitoneal	0.70	0.60 to 0.81	< .001	0.76	0.58 to 0.98	.038
Neck	0.072	0.027 to 0.20	< .001	0.13	0.030 to 0.53	.005
Thoracic	0.11	0.074 to 0.15	< .001	0.16	0.083 to 0.31	< .001
Pelvic	0.10	0.044 to 0.23	< .001	0.14	0.033 to 0.57	.006
Other	0.44	0.33 to 0.58	< .001	0.39	0.26 to 0.60	< .001
Nonadrenal	1	Ref	Ref	1	Ref	Ref
Adrenal	2.47	2.16 to 2.82	< .001	2.09	1.67 to 2.63	< .001
Nonthoracic	1	Ref	Ref	1	Ref	Ref
Thoracic	0.14	0.096 to 0.20	< .001	0.20	0.11 to 0.39	< .001

Abbreviations: INSS, International Neuroblastoma Staging System; OR, odds ratio; Ref, reference.

as stratification variables, including age, stage, and *MYCN* status. All statistical analyses were performed using STATA, version 13 (STATA, College Station, TX).

RESULTS

Clinical and Biologic Features Differ by Primary Tumor Site

The clinical and biologic characteristics at diagnosis of the 8,369 patients in the INRG analytic cohort with an assigned primary tumor site are listed in Table 1, and include 47% with adrenal, 24% with abdominal/retroperitoneal, 15% with thoracic, 3% with pelvic, 3% with neck, and 8% with other primary tumor sites. Each of the evaluated clinical and biologic features showed statistically significant differences when compared across all six primary site categories ($P <$ .001 for all comparisons; Table 1). The most prominent differences (> 10% difference) seemed to be a lower proportion of patients with stage 4 disease, elevated ferritin, elevated LDH, *MYCN* amplification, LOH/aberration at 1p, gain of 17q, 11q aberration, pooled segmental chromosomal aberrations at these loci, and unfavorable International Neuroblastoma Pathology Classification category in thoracic, neck, and pelvic primary tumor sites compared with adrenal primary tumors (Table 1).[11] We also assessed the frequency of International Neuroblastoma Staging System stage 3 tumors across primary sites (n = 1,440 stage 3 tumors in total). Pelvic tumors had the highest frequency of stage 3 disease (41% of pelvic tumors were stage 3), followed by abdominal/retroperitoneal (28%), other (19%), thoracic (18%), adrenal (11%), and neck (10%) sites.

To evaluate some of these differences more closely, we compared features according to the group site variables: adrenal versus nonadrenal and thoracic versus nonthoracic (Table 2). Patients with adrenal tumors had statistically significantly higher proportions of most unfavorable risk factors compared with patients with nonadrenal tumors. In contrast, patients with thoracic tumors had statistically significantly lower proportions of most unfavorable risk factors compared with patients with nonthoracic tumors. Interestingly, there was a higher proportion of thoracic tumors observed in the earlier era (before 1996) compared with more recently diagnosed patients.

Given the striking differences in the incidence of *MYCN* amplification between primary tumor sites, we used logistic regression analysis to assess whether these differences were independent of differences in other features associated with *MYCN* amplification, including age, stage, and grade of differentiation (Table 3). Adrenal primary tumors had double the odds of having *MYCN* amplification compared with nonadrenal primary tumors after controlling for these potential confounders (adjusted odds ratio, 2.09; 95% CI, 1.67 to 2.63; $P <$ .001). Conversely, thoracic primary tumors had one fifth the odds of having *MYCN* amplification compared with nonthoracic primary tumors (adjusted odds ratio, 0.20; 95% CI, 0.11 to 0.39; $P <$ .001).

We also evaluated whether metastatic pattern differs according to primary tumor site. Of the 3,298 patients with stage 4 disease, only 2,899 patients had documented site(s) of metastases in the INRG database and were included in this analysis (Appendix Table 1, online only). Only incidence rates of bone marrow, bone, liver, and "other" metastatic sites showed statistically significant differences across all six primary tumor categories. Specifically, the highest proportion of metastases to the bone marrow (77%), bone (65%), and liver (20%) originated from adrenal primary tumor sites. Bone marrow metastases were also common in patients with abdominal/retroperitoneal (72%) and pelvic (71%) stage 4 tumors. Patients with neck, pelvic, and thoracic stage 4 tumors had lower rates of bone metastasis. Patients with adrenal or abdominal/retroperitoneal metastatic tumors were more likely to have liver metastasis compared with patients with other primary sites.

EFS and OS Differ According to Primary Tumor Site

We next evaluated potential differences in EFS and OS according to primary tumor site. Log-rank tests detected statistically significant differences in times to both outcomes according to primary tumor site. The unadjusted 5-year EFS and OS rates according to the six primary tumor sites were as follows (± SE): adrenal, 56% ± 0.8% and 62% ± 0.8%; abdominal/retroperitoneal, 64% ± 1.1% and 72% ± 1.1%; neck, 79% ± 2.8% and 90% ± 2.2%; thoracic, 80% ± 1.2% and 88% ± 1.0%; pelvic, 81% ± 2.6% and 91% ± 2.0%; and other, 63% ± 2.2% and 70% ± 2.2%, respectively (Figs 1A and 1B; $P <$ .001). Evaluating adrenal versus nonadrenal tumors, we again observed statistically significant differences; the unadjusted 5-year EFS and OS rates were significantly lower for adrenal (estimates previously given) versus nonadrenal primary tumors (EFS and OS for nonadrenal,

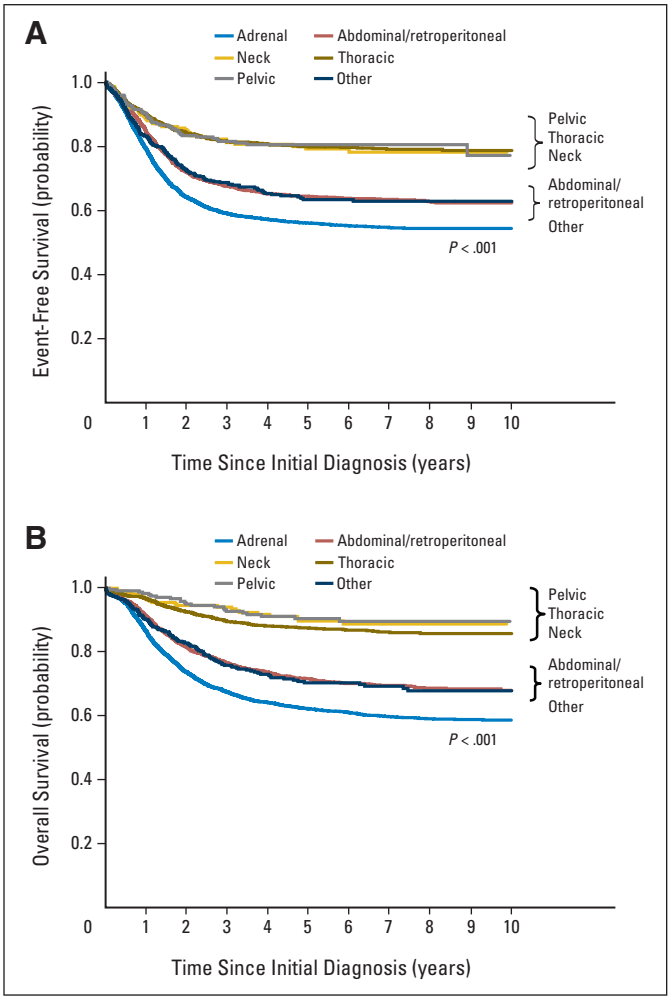

Fig 1. Kaplan-Meier estimated (A) event-free survival and (B) overall survival from time of diagnosis according to primary tumor site.

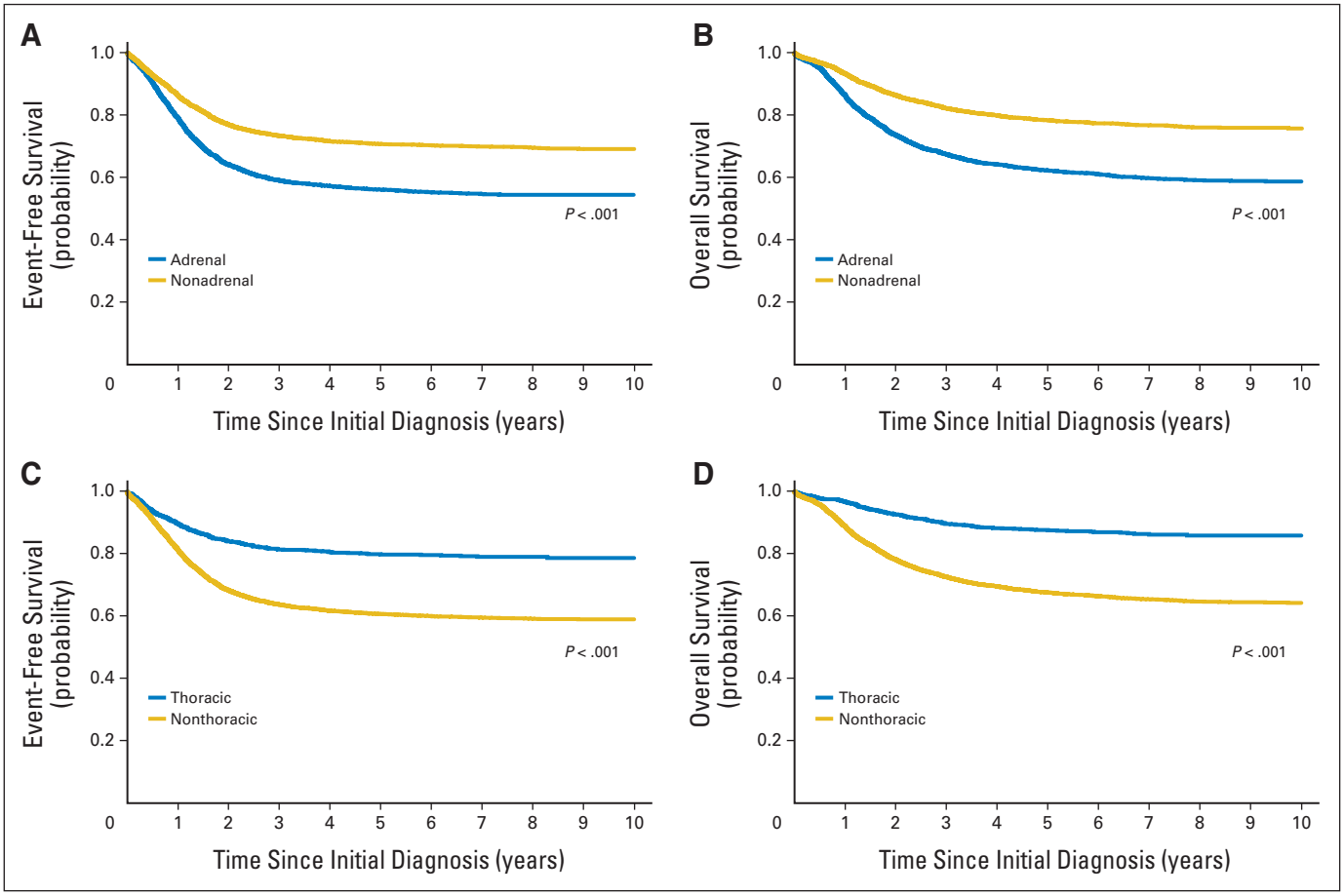

Fig 2. Kaplan-Meier estimated (A) event-free survival and (B) overall survival from time of diagnosis for patients with adrenal versus nonadrenal primary tumor sites. Kaplan-Meier estimated (C) event-free survival and (D) overall survival from time of diagnosis for patients with thoracic versus nonthoracic primary tumor sites.

71% ± 0.7% and 78% ± 0.7%; $P < .001$; Figs 2A and 2B). The opposite was true for thoracic (estimates previously given) versus nonthoracic primary tumors (EFS and OS for nonthoracic, 61% ± 0.6% and 68% ± 0.6%; $P < .001$; Figs 2C and 2D).

Our finding that the three main prognostic factors in NB (age, stage, and *MYCN* status) also differed significantly according to primary tumor site raised the possibility that these differences confounded our univariable observation of differential EFS and OS according to primary tumor site. We therefore constructed Cox proportional hazards models to control for these differences in age, *MYCN* status, and stage (Table 4). In a model evaluating EFS in all six primary tumor sites, only patients with thoracic tumors remained at a decreased risk for an event compared with the reference group of patients with adrenal tumors (adjusted HR, 0.76; 95% CI, 0.64 to 0.89; $P = .001$). Using a similar model for OS, patients with thoracic (adjusted HR, 0.65; 95% CI, 0.52 to 0.80; $P < .001$) or neck (adjusted HR, 0.54; 95% CI, 0.34 to 0.94; $P = .029$) primary tumor sites were at decreased risk for death compared with patients with adrenal tumors. In similar models evaluating adrenal versus nonadrenal tumors, patients with adrenal tumors remained at increased risk for event (adjusted HR, 1.13; 95% CI, 1.03 to 1.23; $P = .008$) and death (adjusted HR, 1.17; 95% CI, 1.05 to 1.29; $P = .003$) compared with patients with nonadrenal tumors. Conversely, patients with thoracic tumors remained at decreased risk for event (HR, 0.79; 95% CI, 0.67 to 0.92; $P = .003$) and death (adjusted HR, 0.68; 95% CI, 0.56 to 0.84; $P < .001$) compared with patients with nonthoracic tumors.

DISCUSSION

In this large comprehensive analysis of primary tumor site in NB, we observed that the primary tumor site may influence some of the heterogeneity in the clinical features, tumor biology, and clinical outcomes in NB. We found statistically significant differences in clinical and biologic characteristics between primary tumor sites. We also observed that patients with primary adrenal tumors had inferior EFS and OS independent of age at diagnosis, *MYCN* status, and International Neuroblastoma Staging System stage. This is in contrast to patients with primary thoracic tumors, who had superior EFS and OS when controlling for these same variables.

Our findings that clinical and biologic features differ according to primary tumor site confirm and extend previous observations. For example, our findings that adrenal tumors are associated with unfavorable prognostic features were also shown in previous smaller studies.[4,5] Likewise, other groups have shown that thoracic primary tumors are associated with younger age, *MYCN* nonamplified tumors, hyperdiploid tumors, and normal LDH and ferritin values.[15,16] To

Table 4. Cox Proportional Hazards Regression Analysis of the Association Between Primary Tumor Site and Event-Free and Overall Survival Without (unadjusted HR) and With Adjustment (adjusted HR) for Age at Diagnosis, *MYCN* Status, and INSS Stage

	Unadjusted			Adjusted		
Primary Tumor Site	HR	95% CI	*P*	HR	95% CI	*P*
			Event-Free Survival			
Adrenal	1	Ref	Ref	1	Ref	Ref
Abdominal/retroperitoneal	0.75	0.69 to 0.83	< .001	0.94	0.85 to 1.04	.225
Neck	0.40	0.30 to 0.54	< .001	0.98	0.70 to 1.36	.886
Thoracic	0.39	0.34 to 0.45	< .001	0.76	0.64 to 0.89	.001
Pelvic	0.39	0.29 to 0.51	< .001	0.89	0.64 to 1.24	.503
Other	0.75	0.65 to 0.87	< .001	0.85	0.72 to 1.02	.079
Nonadrenal	1	Ref	Ref	1	Ref	Ref
Adrenal	1.67	1.55 to 1.80	< .001	1.13	1.03 to 1.23	.008
Nonthoracic	1	Ref	Ref	1	Ref	Ref
Thoracic	0.46	0.40 to 0.52	< .001	0.79	0.67 to 0.92	.003
			Overall Survival			
Adrenal	1	Ref	Ref	1	Ref	Ref
Abdominal/retroperitoneal	0.70	0.63 to 0.77	< .001	0.94	0.84 to 1.06	.313
Neck	0.21	0.14 to 0.33	< .001	0.54	0.31 to 0.94	.029
Thoracic	0.28	0.24 to 0.33	< .001	0.65	0.52 to 0.80	< .001
Pelvic	0.20	0.13 to 0.30	< .001	0.65	0.39 to 1.07	.090
Other	0.70	0.59 to 0.83	< .001	0.86	0.70 to 1.06	.157
Nonadrenal	1	Ref	Ref	1	Ref	Ref
Adrenal	1.97	1.81 to 2.14	< .001	1.17	1.05 to 1.29	.003
Nonthoracic	1	Ref	Ref	1	Ref	Ref
Thoracic	0.34	0.29 to 0.40	< .001	0.68	0.56 to 0.84	< .001

Abbreviations: HR, hazard ratio; INSS, International Neuroblastoma Staging System; Ref, reference.

our knowledge, our finding that more thoracic tumors were diagnosed before 1996 is novel, and may reflect the impact of earlier NB screening efforts that identified a higher proportion of patients with favorable disease.[17,18] Previous studies have shown that the pelvic primary site in NB is a favorable location, with lower rates of *MYCN* amplification and advanced stage than nonpelvic primary tumor sites.[6,7] Given the rarity of occurrence, previous studies of primary NBs of the neck and cervical region are limited to small case series that suggest that favorable clinical and biologic features are also associated with these tumors, including lower-stage disease and less *MYCN* amplification.[19,20] To date, there are no indications that molecular events involved in tumorigenesis are distinct in NB according to primary site, although no genomic studies comparing DNA mutations by primary site have been performed.

It is known that *MYCN*-amplified NBs are characterized by highly aggressive behavior with unfavorable outcome. Perhaps the most striking biologic difference observed in the current study was in the proportion of *MYCN* amplification across primary tumor sites. Although other groups have reported such differences, we show for the first time, to our knowledge, that the substantially different rates of *MYCN* amplification according to primary tumor site are independent of other factors associated with *MYCN* amplification, including age, stage, and grade of differentiation. It is not clear whether developing neuroblasts in the adrenal medulla might be more susceptible to amplification at the *MYCN* locus or if our findings simply reflect a greater number of cells at risk for undergoing *MYCN* amplification at that site because of its size compared with other sympathetic tissues.

We also confirmed previous smaller analyses that demonstrated differences in outcomes according to primary site. For example, the superior unadjusted EFS and OS rates of thoracic primary tumors found in this study (80.0% and 87.6%, respectively) are comparable with those seen in single-center or cooperative group studies with overall survival rates ranging from 71.2% to 100%.[8,15,16,21] The biologically favorable profile of thoracic tumors may explain the better prognosis in these tumors. In one study, *MYCN*-amplified tumors with a thoracic primary were shown to have a better outcome compared with all nonthoracic NB tumors in a previous univariable survival analysis; however, a multivariable Cox analysis was not conducted in that study.[16] A key advantage of our study is our ability to control for potential confounding variables that might be associated both with primary tumor site and prognosis. As the largest multivariable analysis addressing the prognostic impact of primary tumor site, our study demonstrated that the inferior outcomes for patients with adrenal tumors and the superior outcomes for patients with thoracic and neck tumors are independent of differences in age, stage, and *MYCN* status associated with these sites. Interestingly, patients with neck tumors were at decreased risk of death, but not at decreased risk of an analytic event. One reason for this finding may be that, whereas cervical and cervicothoracic NBs have favorable prognostic features, their anatomic localization makes it difficult to completely resect them in many patients, and these tumors tend to recur locally.[19,22-24]

We have confirmed that primary tumor site plays an important role in the heterogeneity of NB. To address our aims, we used the INRG database to evaluate the largest available cohort of patients with NB. However, there are several limitations to analyzing data from a tumor registry. We were limited to the available variables in the registry. As such, we were unable to report on important variables, such as extent of surgery and chemotherapy/radiation treatment used. Although we were able to describe the frequency of stage 3 disease by primary site, we were not able to assess whether a patient was deemed

to have stage 3 disease because of tumor crossing the midline, contralateral node involvement, or both. In addition, we were unable to confirm the primary tumor site designation of the "other" category, which may represent rare primary tumor sites, multifocal primary tumors, large tumors that may cross two or more anatomic compartments, or tumors of unknown origin. Along these lines, some large so-called abdominal/retroperitoneal tumors may have had ambiguous origins and may have actually arisen from adjacent adrenal or pelvic sites. It should be noted that the INRG database contains data from multiple cooperative groups, and it is possible that some patients who were included in our analysis were reported in previous studies on this topic. In addition, contributing cooperative groups may have used slightly different definitions of sites of disease. Moreover, our classification of segmental chromosomal aberrations included only the LOH/aberration at 1p, gain of 17q, and 11q aberration. Our evaluation of these variables separately and also as a pooled variable may differ from previous smaller studies, but these genetic aberrations have been shown to have prognostic significance in previous INRG studies.[12,13] Finally, some clinical and biologic variables were missing for some patients, as noted in Tables 1 and 2, and we could only report on what was available in the INRG database.

On the basis of our findings, we conclude that there are statistically significant differences in clinical features, biologic characteristics, and outcomes between primary tumor sites in NB. Our results suggest that there is something distinctive about the tumor in these specific sites of origin that leads to or reflects different biology and clinical behavior. Further study of the developmental biology of the neural crest and sympathetic nervous system may elucidate the etiology for these observed differences. Likewise, additional efforts should be directed at elucidating the disordered mechanisms of embryonal tumorigenesis in NB.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Support

Supported in part by a Cancer Research UK Life Chair and Programme grant included within a Cancer Research UK Institute of Cancer Research (ICR) Core Award (No. C347/A15403; A.D.J.P.), the National Institute for Health Research Royal Marsden/ICR Biomedical Research Centre (A.D.J.P.), Alex's Lemonade Stand Foundation (K.T.V., K.K.M., S.G.D.), the Frank A. Campini Foundation (K.K.M., S.G.D.), the Edward Conner

Fund (K.K.M.), the Dougherty Foundation (K.K.M., S.G.D.), and the Mildred V. Strouss Chair (K.K.M.). The International Neuroblastoma Risk Group database is supported in part by the William Guy Forbeck Research Foundation, the Little Heroes Cancer Research Fund, the Children's Neuroblastoma Cancer Foundation, the Neuroblastoma Children's Cancer Foundation, and the Super Jake Foundation.

GLOSSARY TERMS

Cox proportional hazards regression model: a statistical model for regression analysis of censored survival data, examining the relationship of censored survival distribution to one or more covariates. This model produces a baseline survival curve, covariate coefficient estimates with their standard errors, risk ratios, 95% CIs, and significance levels.

event-free survival: calculated from the date of diagnosis to the date of the first event, which is resistance, relapse, death, or second malignant neoplasm.

logistic regression analysis: a multivariable regression model in which the log of the odds of a time-fixed outcome event (eg, 30-day mortality) or other binary outcome is related to a linear equation.

loss of heterozygosity (LOH): a situation in which one chromosome has a normal allele of a gene and one chromosome has a mutant or deleted allele.

MYCN: gene encoding for c-myc.

overall survival: the duration between random assignment and death.

Appendix

Table A1. Sites of Metastases of Patients With Stage 4 Neuroblastoma by Primary Tumor Site (n = 2,899)

			Primary Tumor Site												
	All (n = 2,899)		Adrenal (n = 1,773)		Abdominal/ Retroperitoneal (n = 628)		Neck (n = 38)		Thoracic (n = 258)		Pelvic (n = 34)		Other (n = 171)		
Metastatic Site	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	*P**
Bone marrow	2,146	74	1,362	77	455	72	24	63	166	64	22	71	117	68	< .001
Bone	1,783	61	1,158	65	361	57	16	42	132	51	14	45	102	60	< .001
Distant lymph nodes	1,000	34	635	36	199	32	14	37	85	33	9	29	58	34	.499
Liver	514	18	359	20	100	16	3	8	27	10	3	10	22	13	< .001
Lung	99	3	68	4	22	3	0	0	3	1	1	3	5	3	.269
CNS	76	3	55	3	10	2	2	5	4	2	0	0	5	3	.199
Skin	80	3	48	3	15	2	1	3	7	3	0	0	9	5	.390
Other	820	28	520	29	143	23	11	29	72	28	9	29	65	38	.003

*P value refers to χ^2 test.