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Neuroblastoma: the association of anatomical tumour site, molecular biology and patient outcomes

Adeline Salim,* Arimatias Raitio,*† Barry Pizer,‡ Dhanya Mullassery* and Paul D. Losty*§

*Department of Paediatric Surgery, Alder Hey Children's Hospital NHS Foundation Trust, Liverpool, UK
†Department of Paediatric Surgery, Turku University Hospital and The University of Turku, Turku, Finland
‡Department of Oncology, Alder Hey Children's Hospital NHS Foundation Trust, Liverpool, UK and
§Institute of Child Health, School of Health and Life Sciences, –The University of Liverpool, Liverpool, UK

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Correspondence
Professor Paul D. Losty Department of Paediatric Surgery, Alder Hey Children's Hospital NHS Foundation Trust, Eaton Road, Liverpool L12 2AP, UK. Email: paul.losty@liv.ac.uk

A. Salim MBChB, MRCS; **A. Raitio** MD, PhD; **B. Pizer** PhD, FRCPCH; **D. Mullassery** PhD, FRCS (Paed); **P. D. Losty** MD, FRCSI, FRCS (Ed), FRCS (Eng), FRCS (Paed), FEBPS.

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Abstract

Background: Numerous factors have been identified as carrying prognostic value in neuroblastoma (NB) and therefore incorporated in risk stratification of disease. Here, we investigate the association of anatomical site of NB with molecular biology and clinical outcomes.
Methods: A total of 117 patients with NB were studied over a 30-year period. Tumour location was confirmed with computed tomography/magnetic resonance imaging. Data on molecular biology were obtained as testing became available. Chi-squared, Fisher's exact test and Kaplan–Meier log-rank tests were used for statistical analysis.
Results: Tumour originated in the thoracic region (thoracic NB, TNB) in 15 patients (13%), adrenal gland (adrenal NB, ANB) in 88 patients (75%) and abdominal/paravertebral chain (paravertebral NB, PVNB) in 14 patients (12%). Overall survival (OS) for ANB was significantly lower (38%; $P = 0.015$). ANB cases were more frequently diagnosed at stage IV (69%; $P = 0.001$). MYCN amplification was noted in 33% of ANB cases and associated with lower OS (17% versus 62% MYCN non-amplified ANB; $P = 0.01$). The vast majority of TNB and PVNB were non-MYCN amplified (100% and 86%, respectively) and carried better prognosis (OS 86% and 83%, respectively). Forty-two percent of ANB cases were diploid and had lower OS (20% versus 71% hyperdiploid ANB; $P = 0.079$). TNB and PVNB were found to be mostly hyperdiploid (86% and 100%, respectively) with better OS (83% and 33%, respectively). Segmental chromosomal alterations had prognostic significance in those with PVNB ($P = 0.03$).
Conclusion: TNB tumours have better outcomes than adrenal tumours. This may be due to varied factors reported here including non-metastatic disease at presentation, non-amplification of the MYCN oncogene and overall favourable molecular biology characteristics.

Introduction

Neuroblastoma (NB) is the most common extracranial solid tumour in infants and children accounting for 8–10% of all malignant childhood tumours.[1] The most common tumour sites include abdomen (adrenal gland 48% and extra-adrenal retroperitoneum 25%) and thorax (16%) but NBs can also rarely arise from the pelvis (3%) and the neck (3%).[2] Numerous factors have been identified as carrying prognostic value in NB, many of which have been incorporated into various classification or staging systems.[2–4] The currently accepted International Neuroblastoma Risk Group staging system characterizes pre-treatment tumours according to the absence or presence of multiple image-defined risk factors and genetic characteristics of the tumour further stratified patients into low-/high-risk groups.[2]

Amplification of MYCN oncoprotein is strongly associated with rapid disease progression and poor outcome in patients independent of age and tumour staging.[4,5] Other chromosomal aberrations associated with either whole DNA copy number alterations or incomplete segmental alterations have also been shown to predict NB behaviour. In particular, aggressive tumour behaviour and poor outcome are associated with deletions at the chromosomal region 1p36.3[6] or 11q23,[7] and with unbalanced gain of the long arm of chromosome 17 (17q21 to 17qter).[8] Tumour cell DNA content in

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NB falls into two main categories: near-diploidy or hyperdiploidy (e.g. triploidy) – in patients younger than 18 months with metastatic disease, a near-diploid DNA content is a predictor of poor outcome.[9,10] More recently, mutations in several genes such as *ALK*, *TERT*, *ATRX* and *PTPRD* have been implicated in the outcome of NB patients[11] and authors have thus also suggested incorporating these novel biomarkers into existing risk prognostication system.[12]

Several studies have reported that neuroblastic tumours originating from different anatomical sites follow diverse clinical outcomes.[13–18] Nevertheless, it is unclear here whether the tumour site alone carries prognostic significance or whether any survival benefit is due to the biological and molecular characteristics of the tumour cells. There is limited evidence available which has directly compared characteristics and clinical outcomes of abdominal and extra-abdominal NBs.[19,20] Therefore, in this current study, we aim to further investigate the association between anatomical site of NB and their clinical, biological and molecular characteristics with resultant clinical outcomes.

Table 1 The 5-year OS. Adrenal neuroblastoma has significantly worse outcomes

Tumour location	Number of patients	5-year OS (%)	*P*-value
Thoracic	15	11 (73)	0.01
Paravertebral	14	6 (43)	
Adrenal	88	33 (38)	

OS, overall survival.

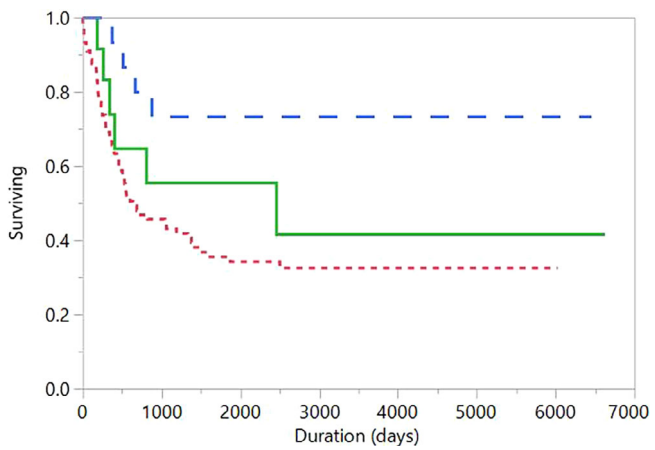

Fig 1. Overall survival of neuroblastoma (------, abdomen – adrenal; ——, abdomen – paravertebral; - - - - -, thorax).

Methods

We undertook a retrospective analysis of all children diagnosed with NB between 1985 and 2013 identified from our institution's oncology database. One hundred and seventeen patients were identified, and tumour location was confirmed with computed tomography/magnetic resonance imaging. Data on molecular biology were obtained as testing became available. Chi-squared, Fisher's exact test and Kaplan–Meier log-rank tests were used for statistical/survival analysis. A significance level of $P \leq 0.05$ (two-tailed) was set. Analyses were performed using JMP Pro, version 13.1.0 for Windows (SAS Institute Inc., Cary, NC, USA). Study was approved by the Department of Oncology and Pathology, Alder Hey Children's Hospital, Liverpool, UK. Ethical approval was not needed as this article does not contain any studies with human participants or animals performed by any of the authors.

Results

Tumour site and outcome

Fifteen patients (13%) had thoracic tumours (thoracic NB, TNB), 14 (12%) abdominal/paravertebral chain tumours (paravertebral NB, PVNB) and 88 (75%) adrenal gland tumours (adrenal NB, ANB) (Table 1). ANB cases had significantly poorer outcome compared to all other anatomical groups (Fig. 1). The majority of ANB tumours were diagnosed as International Neuroblastoma Staging System(INSS) stage IV (Table 2). The median age at diagnosis was 2 years (3 days–14 years). No significant association was found with anatomical tumour origin and age at diagnosis ($P = 0.198$).

Genetic analysis

MYCN status was available for 52 patients: amplification was noted in 33% of ANB cases and associated with significantly lower 5-year overall survival (OS, 17%) compared to MYCN non-amplified ANB (62%; $P = 0.01$). Where MYCN data were available, all TNB cases were MYCN non-amplified (5-year OS 86%) and all but one PVNB cases (86%) also showed no MYCN amplification (5-year OS 83%) (Table 3). Comparing MYCN non-amplified cases ($n = 39$) against tumour site, we observed that the 5-year OS was also lowest in ANB (62%) but no statistically significant differences were observed comparing TNB and PVNB cases (5-year OS 86% and 83%, respectively; $P = 0.33$).

DNA copy data were available for 22 cases: 42% of ANB tumours were diploid and associated with reduced 5-year OS (20% versus 71% hyperdiploid ANB; $P = 0.079$). In contrast, the majority of TNB and PVNB tumours were hyperdiploid (86% and 100%,

Table 2 Staging at diagnosis. Adrenal neuroblastoma was significantly more often diagnosed in stage IV

	Stage I	Stage II	Stage III	Stage IV	Stage IVs	*P*-value
Thoracic	0	6	3	6	0	0.001
Paravertebral	0	3	3	8	0	
Adrenal	3	2	12	61	10	

Table 3 MYCN amplification in adrenal NB and chromosomal alterations in paravertebral NB are associated with significantly worse outcome

Tumour site	MYCN amplification	Number of patients	5-year OS (%)	*P*-value	17q gain and/or 1p/11q deletion	Number of patients	5-year OS (%)	*P*-value
Thoracic	No	7	86	N/A	No	3	100	0.88
	Yes	0	N/A		Yes	1	0	
Paravertebral	No	6	83	0.28	No	3	100	0.03
	Yes	1	0		Yes	2	0	
Adrenal	No	26	62	0.01	No	5	20	0.60
	Yes	12	17		Yes	14	43	

N/A, data not available; NB, neuroblastoma; OS, overall survival.

Table 4 Multivariable regression analysis showing that only age at diagnosis and MYCN status are significantly associated with patient outcomes

	P-value
Age at diagnosis	0.001
MYCN amplification	0.014
Stage	0.19
Tumour location	0.58

respectively) and associated with improved 5-year OS (83% for TNB, $P = 0.088$ and 33% for PVNB).

Segmental chromosomal alterations (17q gain, 1p/11q deletions) were detected in 17 of 28 patients and had prognostic significance in those with PVNB (Table 3).

Statistical modelling

We performed Cox proportional hazards regression analysis to fully investigate the associations between tumour site, age at diagnosis, MYCN status and tumour stage with 5-year OS. In our study series, only MYCN ($P = 0.014$) and age at diagnosis ($P = 0.001$) were identified to be independent prognostic factors (Table 4).

Discussion

The current study has shown statistically significant relationships between NB tumour site, their genetic characteristics and clinical outcome(s). Historical studies have previously suggested that thoracic neuroblastic tumours may be associated with better overall prognosis.[14,17,21] These observations have now been further reinforced by more contemporaneous work which has compared NB tumour site(s) with prognostic factors such as histology, MYCN status and biochemical markers.[15,19,22]

Our findings have herein demonstrated that TNB has significantly better outcome(s) than ANB (Table 1) and that these patients are more likely to present with only locoregional disease (INSS stage II–III) compared to ANB lesions (Table 2). TNB tumours likewise tended to exhibit favourable molecular biology profile(s), namely: negative MYCN amplification, negative segmental chromosomal alterations and DNA index >1 (Table 3).

Previously held consensus has identified TNB as a distinct disease subset that presents at earlier age.[17,23] We have found in this study no difference(s) in presenting age in our population which is also in keeping with findings from recent works.[15,19]

The underlying mechanism(s) as to why TNB tumours have better survival outcome than ANB and why the thoracic location of the lesion itself confers independent prognostic value is subject to much debate. Multivariate analyses from a number of multicentre retrospective studies have shown conflicting findings.

Data from the Pediatric Oncology Group published by Morris *et al*.[17] showed that the thoracic location of tumour confers survival advantage(s) independent of DNA index, MYCN status and serum lactate dehydrogenase levels. This finding is also supported by a report from the International Neuroblastoma Risk Group[24] that demonstrated thoracic tumours had a lower hazard ratio compared to non-thoracic tumours after adjusting for patient age, MYCN status and stage of disease.

However, data from the German Cooperative Study Group NB90[15] showed that only tumour stage, MYCN status and serum lactate dehydrogenase were independent prognostic factors and not the location of tumour. In our current study, we herein report only MYCN status and age at primary diagnosis as independent prognostic factors and not the tumour location itself. This implies that the OS advantage of TNB over ANB is due to the inherent characteristics of TNB tumours rather than the anatomical location alone. These results also confirm our previous observation from a smaller cohort of patients.[25] Our findings are limited by sample size and availability of genetic analysis, nevertheless they corroborate well with other larger studies.[11]

There is growing evidence in NB that genetic and molecular differences exist resulting in the fascinating and enigmatic behaviour of this tumour. Cooper *et al*. have shown that NB cells can 'arrest' at various levels of adrenal medullary cell differentiation and that a process of differentiation/dedifferentiation maybe responsible for the biological 'switch' from malignant to benign tumour phenotype in some cases of NB.[26] This intriguing hypothesis may also usefully be supported by *in vivo* laboratory work from our science group in the chick embryo NB model, which has demonstrated evidence of cell differentiation, reduced cell division and undetectable MYCN expression in MYCN-amplified NB cells implanted in the avian system that then migrate into the sympathetic ganglia. In non-neural locations, the implanted MYCN NB cells in the chick continued to rapidly proliferate aggressively and overexpress MYCN.[27]

NB is therefore a 'molecular defined disease' greatly influenced by the genetic properties of the tumour cell.[11,28–32] NB tumours at

specific anatomical sites likely derive from a very distinct embryological milieu associated with unique genetic profiling and survival outcome(s). Future research should therefore be vigorously directed to encompass complete genetic and molecular biology profiling of neuroblastic tumours.

Conclusion

TNB tumours have better overall outcome(s) than primary adrenal neoplasms. This may be due to varied factors reported here including non-metastatic disease at presentation, non-amplification of the MYCN oncogene and favourable molecular biology.

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Author Contributions

Adeline Salim: Conceptualization; data curation; formal analysis; investigation; methodology; writing-original draft; writing-review and editing. **Arimatias Raitio:** Conceptualization; formal analysis; investigation; methodology; writing-original draft; writing-review and editing. **Barry Pizer:** Conceptualization; data curation; methodology; resources; supervision; writing-review and editing. **Dhanya Mullassery:** Conceptualization; data curation; methodology; writing-review and editing. **Paul D. Losty:** Conceptualization; methodology; project administration; resources; supervision; writing-original draft; writing-review and editing.

Conflicts of interest

None declared.

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