**NEUROBLASTOMA: THE ASSOCIATION OF ANATOMICAL TUMOUR SITE, MOLECULAR BIOLOGY AND PATIENT OUTCOMES**

Running head: Neuroblastoma and patient outcomes

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**Abstract**

**Background**

Numerous factors have been identified as carrying prognostic value in neuroblastoma and therefore incorporated in risk stratification of disease. Here we investigate the association of anatomical site of neuroblastoma with molecular biology and clinical outcomes.

**Methods**

117 patients with neuroblastoma were studied over a 30-year period. Tumour location was confirmed with CT/MRI imaging. Data on molecular biology was obtained as testing became available. Chi-square, Fisher’s exact test and Kaplan-Meier Log-Rank tests were used for statistical analysis.

**Results**

Tumour originated in the thoracic region (TNB) in 15 patients (13%), adrenal gland (ANB) in 88 patients (75%), and abdominal/paravertebral chain (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% ANB and associated with lower OS (17% vs 62% MYCN non-amplified ANB; p=0.01). The vast majority of TNB and PVNB were non-MYCN amplified (100% and 86%) and carried better prognosis (OS 86% and 83%). 42% ANB cases were diploid and had lower OS (20% vs 71% hyperdiploid ANB; p=0.079). TNB and PVNB were found to be mostly hyperdiploid (86% and 100%) with better OS (83% and 33%, respectively). Segmental chromosomal alterations had prognostic significance in those with PVNB (p=0.03).

**Conclusion**

Thoracic neuroblastoma 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.

**Keywords**

Neuroblastoma

Patient outcomes

Molecular biology

MYCN

1. **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%, 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 neuroblastoma, many of which have been incorporated into various classification or staging systems.2-4 The currently accepted International Neuroblastoma Risk Group (INRG) staging system characterises pre-treatment tumours according to the absence or presence of multiple image-defined risk factors (IDRF) 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 neuroblastoma behaviour. In particular, aggressive tumour behaviour and poor outcome are associated with deletions at the chromosomal region 1p36.36 or 11q237, and with unbalanced gain of the long arm of chromosome 17 (17q21 to 17qter)8. Tumour cell DNA content in neuroblastoma falls into 2 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 neuroblastoma patients11 and authors have thus also suggested incorporating these novel biomarkers into existing risk prognostication system12.

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 neuroblastomas.19,20 Therefore, in this current study, we aim to further investigate the association between anatomical site of neuroblastoma and their clinical, biological, and molecular characteristics with resultant clinical outcomes.

1. **Methods**

We undertook a retrospective analysis of all children diagnosed with neuroblastoma between 1985-2013 identified from our institution’s oncology database. One hundred and seventeen patients were identified, and tumour location was confirmed with CT/MRI imaging. Data on molecular biology was obtained as testing became available. Chi-square, Fisher’s exact test and Kaplan-Meier Log-Rank tests were used for statistical/survival analysis. A Significance level of p≤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 Department of Oncology and Pathology, Alder Hey Children’s Hospital, Liverpool, UK.

1. **Results**

**3.1 Tumour site and outcome**

Fifteen patients (13%) had thoracic tumours (TNB), 14 (12%) abdominal/paravertebral chain tumours (PVNB), and 88 (75%) adrenal gland tumours (ANB) (Table 1). ANB cases had significantly poorer outcome compared to all other anatomical groups (Figure 1). The majority of ANB tumours were diagnosed as 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).

**3.2 Genetic analysis**

MYCN status was available in 52 patients: amplification was noted in 33% ANB and associated with significantly lower 5-year OS (17%) compared to MYCN non-amplified ANB (62%, p = 0.01). Where MYCN data was 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 5-year OS was also lowest in ANB (62%) but no statistically significant differences were observed comparing to TNB and PVNB (5-year OS 86% and 83% respectively, p=0.33).

DNA copy data was available in 22 cases: 42% ANB tumours were diploid and associated with reduced 5-year OS (20% vs 71% hyperdiploid ANB; p = 0.079). In contrast, the majority of TNB and PVNB tumours were hyperdiploid (86% and 100% 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 17/28 patients and had prognostic significance in those with PVNB (Table 3).

**3.3 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).

1. **Discussion**

The current study has shown statistically significant relationships between neuroblastoma 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 neuroblastoma 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 multi-centre 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 LDH levels. This finding is also supported by a report from the INRG24 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 NB9015 showed that only tumour stage, MYCN status, and serum LDH 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 of itself. This implies that the overall survival 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 neuroblastoma that genetic and molecular differences exist resulting in the fascinating and enigmatic behaviour of this tumour. Cooper et al. have shown that neuroblastoma 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 neuroblastoma.26 This intriguing hypothesis may also usefully be supported by in vivo laboratory work from our science group in the chick embryo neuroblastoma model which has demonstrated evidence of cell differentiation, reduced cell division, and undetectable MYCN expression in MYCN amplified neuroblastoma cells implanted in the avian system that then migrate into the sympathetic ganglia. In non-neural locations, the implanted MYCN neuroblastoma cells in the chick continued to rapidly proliferate aggressively and over express MYCN.27

Neuroblastoma therefore is a ‘molecular defined disease’ greatly influenced by the genetic properties of the tumour cell.11,28-32 Neuroblastoma 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.

1. **Conclusion**

Thoracic neuroblastoma 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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**Disclosure statement**

Conflict of interest: None declared.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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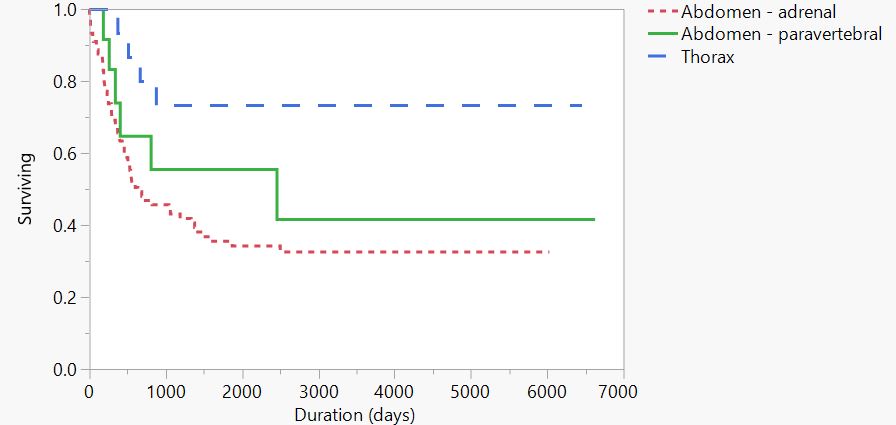
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**Figure 1.** Overall survival of neuroblastoma.



|  |  |  |  |
| --- | --- | --- | --- |
| Tumour Location | Number of Patients | 5-year overall survival (%) | P value |
| Thoracic | 15 | 11 (73) | 0.01 |
| Paravertebral | 14 | 6 (43) |
| Adrenal | 88 | 33 (38) |

**Table 1.** 5-year overall survival. Adrenal neuroblastoma has significantly worse outcomes.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | 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 2.** Staging at diagnosis. Adrenal neuroblastoma was significantly more often diagnosed in stage IV.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Tumour site | MYCN amplification | Number of patients | 5-year overall survival (%) | P value | 17q gain and/or 1p/11q deletion | Number of Patients | 5-year overall survival (%) | 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 |

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

n/a – data not available

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

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