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

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Key words
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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% MYCN non-amplified 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.
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 patients11 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.

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 ≤ 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 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).

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        |         |

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respective) 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).

**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 1q deletion | 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        |

**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 \textit{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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