A nomogram for predicting recurrence-free survival of intermediate and high-risk neuroblastoma

Quan Sun1 · Yanmin Chen1 · Qianya Jin1 · Xiaojun Yuan1

Received: 23 April 2022 / Revised: 25 August 2022 / Accepted: 7 September 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
This study aimed to confirm the independent risk factors for recurrence-free survival (RFS) in intermediate and high-risk neuroblastoma (NB) patients and set up an effective nomogram model for predicting the recurrence of NB. A total of 212 children with intermediate- and high-risk neuroblastoma, who had ever achieved complete remission (CR) or very good partial remission (VGPR) after standardized treatment in this hospital, were chosen as study objects. After retrospective analysis of the clinical data, Cox regression model was used to explore the factors related to the recurrence of neuroblastoma, to determine the variables to construct the Nomogram. The consistency index would predict the accuracy of this nomogram. RFS rate in 1-year, 3-year, 5-year, and 10-year was 0.811, 0.662, 0.639, and 0.604, respectively. Children with MYCN amplification had a higher neuron-specific enolase (NSE) value \((P = 0.031)\) at the initial diagnosis than MYCN non-amplification. The univariate analysis predicted that increased vanillylmandelic acid (VMA) and NSE value and dehydrogenase (LDH) \(>1000\) U/L were important adverse factors for the recurrence of NB. Multivariate analysis demonstrated that age at diagnosis, tumor localization, MYCN state, histologic subtype, and tumor capsule were significantly associated with RFS (all \(P\) values \(<0.05\)). Nomograms were established for predicting the recurrence of NB according to the Cox regression analysis. Internal verification by the Bootstrap method showed that the prediction of the nomogram’s consistency index (C-index) was 0.824 \((P = 0.023)\).

Conclusion: Age at diagnosis, tumor localization, MYCN state, histologic category, and tumor capsule were independent risk factors for the recurrence of NB. The nomogram model could accurately predict the recurrence of children with neuroblastoma.

What is Known:
• The prognoses of neuroblastoma (NB) could vary greatly due to the high heterogeneity, the 5-year survival rate of low-risk NB exceeded 90%, while the 5-year survival rate of children in the intermediate and high-risk groups was not satisfactory.

What is New:
• Increased vanillylmandelic acid (VMA) and neuron-specific enolase (NSE) value, and lactate dehydrogenase (LDH) \(>1000\) U/L were important adverse factors for the recurrence of NB.
• NSE value was more valuable for predicting NB recurrence.

Keywords Recurrence · Neuroblastoma · Recurrence-free survival · MYCN · Nomogram

Introduction
Neuroblastoma (NB) sprang from the multipotent neural crest cells in the sympathetic nervous system. It preferably occurred in the adrenal medulla. It could also be extended to the paravertebral sympathetic nerves of the neck, chest, abdomen, and pelvis [1]. NB was the most typical extracranial solid tumor in childhood, with an incidence rate of about 8–10% of all childhood malignant tumors. Nevertheless,
mortality accounted for 15% of childhood malignant tumor-related deaths [2–4]. Due to the insidious onset of NB, about 40% of children had bone marrow, bone, or other metastases at the first diagnosis, leading to a poor general prognosis [5]. Their prognoses could vary greatly due to the high heterogeneity thereby underlying the above statement. The 5-year survival rate of low-risk NB exceeded 90%, while the 5-year survival rate of children in the intermediate and high-risk groups was not satisfactory [6]. It was still difficult to cure through comprehensive multidisciplinary treatment such as surgery, chemotherapy, and radiotherapy. Recurrence often occurred after complete remission (CR), and the survival rate after recurrence was less than 20% [2, 7]. It was crucial to precisely determine the recurrence by monitoring NB-related indicators and to give timely salvage treatment, especially in the intermediate and high-risk groups.

Clinically, we defined relapse-free survival (RFS) as the duration from CR or very good partial response (VGPR) to recurrence. Chinese Children’s Cancer Group (CCCG) of the China Anti-Cancer Association developed the Multidisciplinary Treatment Guideline for Neuroblastoma. It defined CR that all primary and metastatic tumors disappeared, and the value of catecholamines and metabolites decreased to standard level. It defined VGPR that the primary tumor volume decreased by 90–99%; all measurable metastases disappeared; catecholamines and metabolites got back to normal. VGPR allowed a 99Tc scan of bone lesions to be positive; catecholamines and metabolites got back to normal. It defined VGPR that the primary tumor volume decreased by 90–99%; all measurable metastases disappeared; catecholamines and metabolites got back to normal. Therefore, the study was performed to analyze (a) the clinical features of patients with recurrent NB and (b) the relevant risk factors for RFS and set up a nomogram model to predict RFS in high-risk NB patients, but the related factors affecting RFS needed further study [9]. Many studies reported that the prognostic factors for NB include age, pathological type, International Neuroblastoma Staging System (INSS), MYCN state, etc. [10, 11]. It also reported that the time from diagnosis to the first recurrence could affect survival in recurrent NB patients [7]. The survival rate of recurrent NB was relatively low, so it was significant to monitor recurrence accurately. Therefore, the study was performed to analyze (a) the clinical features of patients with recurrent NB and (b) the relevant risk factors for RFS and guide the follow-up interval after CR or VGPR in this center.

Medical nomograms used clinic data and other variables, such as tumor stage and age, to set up a statistical prediction model. It could predict the probability of recurrence or death for a specific individual [12]. The most significant advantage was that it could assess the risk of clinical events individually according to patient and disease characteristics. The nomogram could incorporate continuous variables and independent risk factors for the disease into the prognosis. It was superior to the clinician in assessing events and had a wide range of applications in various cancers [13–15]. Therefore, we established a predicting nomogram based on the relevant factors affecting the RFS of recurrent NB, which could help clinicians plan the follow-up interval and detect recurrence in time.

**Manuscript formatting**

**Materials and methods**

**Patients**

There were 212 cases of pediatric NB enrolled in this study from January 1, 2007, to December 31, 2018. They were all treated by the Multidisciplinary Treatment Guideline for Neuroblastoma in the Department of Pediatric Hematology/Oncology, Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine. The inclusion criteria for patients were indicated as follows: (a) risk groups were the intermediate-risk group or high-risk group according to Children’s Oncology Group (COG). (b) Patients had achieved CR or VGPR after standardized treatment according to CCCG’s guidelines. (c) Patients were younger than 18 years old at diagnosis. We retrospectively collected clinical characteristics of each patient concerning age, gender, chief complaint, INSS stage, primary site, metastasis site (especially bone and bone marrow metastasis), MYCN state (amplified or not), histologic category, intact tumor capsule (with, without), tumor size (5 cm or less, 5.1–9.9 cm, 10 cm or more), the time-point for reaching CR or VGPR, etc. The value of vanillylmandelic acid (VMA), neuron-specific enolase (NSE), serum ferritin, and lactic dehydrogenase (LDH) before treatment also was recorded. The tumor capsule and tumor size were recorded according to the surgeon’s exploration and measurement during the operation. For the recurrent children, we paid more attention to identifying the recurrent time to determine RFS.

**Diagnosis, pathology, stage, and risk stratification**

Two methods were used to diagnose neuroblastoma in this center. (a) Pathological diagnostic criteria were the gold standard for NB. (b) Imaging, bone marrow puncture, or biopsy suggested suspicious NB, accompanied by a significant increase in urine (or serum) catecholamines or their metabolites. International Neuroblastoma Pathology Classification (INPC) was adopted for the histologic category of neuroblastoma. It included NB (undifferentiated or poorly undifferentiated), NB (differentiated), ganglioneuroblastoma intermixed (GNBi), and ganglioneuroblastoma nodular (GNBn) [16, 17]. Patients were classified by INSS. It defined stage 1, 2A, or 2B, and 4s as the localized tumor; stage 3 as an unresectable infiltrating
tumor; and stage 4 as any primary tumor with dissemination. Finally, patients were divided into the low-risk group, intermediate-risk group, and high-risk group respectively according to age, INSS, INPC, MYCN state, etc., by COG criteria.

**Treatment procedure**

Regarding the CCCG’s guidelines, children in the intermediate-risk group would be treated with alternative surgery before chemotherapy or after (about four cycles), then following chemotherapy till the end of four cycles after CR or VGPR. The total cycles of chemotherapy did not exceed eight. A second surgery could be operated on if necessary. The maintenance treatment was 13-cis-RA 160 mg/m²/day, consecutive 14 days per month, 6 months in total, while cases of the high-risk group were treated with neoadjuvant chemotherapy (about four cycles) followed by selective operation, and then additional four cycles of chemotherapy.

**Table 1** Clinical characteristics of neuroblastoma

| Factors                      | No. (%) | Factors                      | No. (%) |
|------------------------------|---------|------------------------------|---------|
| Gender                       |         | VMA (mg/24 h)                |         |
| Male                         | 131 (61.8) | Normal                       | 118 (55.7) |
| Female                       | 81 (38.2)  | Abnormal                      | 94 (44.3)  |
| Age (months)                 |         | NSE (µg/l)                   |         |
| ≤ 12                         | 40 (18.9)  | Normal                       | 13 (6.1)   |
| 12~18                        | 23 (10.8)  | Abnormal                      | 199 (93.9) |
| ≥ 18                         | 149 (70.3) | LDH(U/L)                     |         |
| INSS                         |         | > 1000                       | 42 (19.8)  |
| 1                            | 8 (3.8)   | ≤ 1000                        | 170 (80.2) |
| 2                            | 32 (15.1) | Serum ferritin (µg/l)        |         |
| 3                            | 60 (28.3) | Normal                       | 62 (29.2)  |
| 4                            | 106 (50)  | Abnormal                      | 150 (70.8) |
| 4s                           | 6 (2.8)   | MYCN state                   |         |
| Primary location             |         | Non-amplification            | 125 (58.9) |
| Retroperitoneum/paranephros  | 166 (78.3) | Amplification                | 45 (21.2)  |
| Mediastinum                   | 33 (15.6) | NA                           | 42 (19.9)  |
| Neck                         | 5 (2.4)   | Histologic category          |         |
| Vertebra                     | 4 (1.9)   | NB (undifferentiated or poorly undifferentiated) | 121 (57.1) |
| Pelvis/sacroccyx             | 4 (1.9)   | NB (differentiated)          | 37 (17.5)  |
| Metastasis location          |         | GNBi                          | 45 (21.2)  |
| None                         | 63 (29.7) | GNBn                          | 9 (4.2)    |
| Bone                         | 89 (42)   | Tumor capsule                |         |
| Lymph node                   | 29 (13.7) | Intact                       | 133 (62.7) |
| Multiple metastases*         | 37 (17.5) | Fractured                    | 77 (36.3)  |
| Bone marrow                  | 82 (38.7) | NA (without surgery)         | 2 (0.9)    |
| Others                       | 14 (6.6)  | Tumor sizes (cm)             |         |
| Chief complaint              |         | ≤ 5                           | 96 (45.3)  |
| Physical examination         | 57 (26.9) | 5.1–9.9                      | 106 (50.0) |
| Asymptomatic mass            | 49 (23.1) | ≥ 10                          | 10 (4.7)   |
| Digestive system symptoms    | 59 (27.8) | Recurrence                   |         |
| Respiratory symptoms         | 10 (4.7)  | Yes                           | 71 (33.5)  |
| Fever                        | 14 (6.6)  | No                            | 141 (66.5) |
| Motor symptoms               | 21 (9.9)  |                              |         |
| others                       | 2 (0.9)   |                              |         |

The normal value of VMA: \(< 2\) mg/24 h (<2 years old), \(< 5\) mg/24 h (2–18 years old); the normal value of NSE: 0–16.3 ng/ml; the normal value of serum ferritin: 20–290 µg/l

NB neuroblastoma, GNBi ganglioneuroblastoma intermixed, GNBn ganglioneuroblastoma nodular, MRD minimal residual disease, LDH lactate dehydrogenase, NES neuron-specific enolase, VMA vanillylmandelic acid

*Multiple metastases (≥ 3 metastases)
after CR or VGPR. This group should be taken in tandem autologous stem cell transplantation and local radiotherapy after conventional chemotherapy. But patients who could not afford stem cell transplantation should take 10 to 12 cycles of chemotherapy in total. The maintenance treatment was the same as the intermediate-risk group.

**Follow-up of patients**

Each patient had a unique portfolio that recorded their treatment response, treatment effect, and follow-up. We looked through their portfolio to find out when they achieved CR or VGPR. If the portfolio was incomplete or the patient did not follow up on schedule, we would call them to complete a brief oral questionnaire through the telephone. At first, all patients were followed up with lab examinations (VMA and NSE) and imaging examinations (CT, MRI, or PET/CT) every two cycles of chemotherapy. After CR or VGPR, they were followed up every 3 to 6 months. Event was defined as recurrence or death for any cause.

**Statistical analysis**

All statistical analysis was performed using SPSS statistical software for Windows, version 19.0 (SPSS, Chicago, IL, USA). The quantitative data were expressed as median (range). The value of VMA, NSE, serum ferritin, and LDH satisfied the non-normal distribution. Wilcoxon rank-sum test was used to show the correlation between MYCN amplification and clinical features of neuroblastoma. The count data were expressed by proportion (%). RFS was analyzed through Kaplan–Meier curves, and the Log-rank test was used for univariate survival analysis to determine clinical factors related to recurrence. Cox proportional hazards model was applied to multivariate analysis to evaluate independent risk factors affecting recurrence. Finally, those risk factors that had been demonstrated statistically significant in univariate survival analysis would be built into a predictive recurrence nomogram. All tests were bilateral, and p-values less than 0.05 were regarded as statistical significance [18].

The nomogram was derived from the results of Cox regression analysis and compiled using the RMS package in R version 3.6.2 (http://www.R-project.org/). One thousand re-sampling Bootstraps were performed for internal verification to reduce over-fitting bias. The consistency index (C-index) was used to evaluate the predictive performance of the nomogram. It represented the probability of consistency between actual recurrence and recurrence predicted from the model. The C-index ranged from 0 to 1. The prediction would be more accurate if the C-index were close to one [19].

**Fig. 1** Kaplan–Meier curve analysis of RFS according to the duration of MRD in the bone marrow from positive to negative. 2cycles represent that MRD in the bone marrow turns from positive to negative after 2 cycles of chemotherapy; the same applies for 4 cycles. MRD, minimal residual disease. HR, hazard ratio

![Kaplan–Meier curve analysis of RFS in children with neuroblastoma.](image)

**Fig. 2** Kaplan–Meier curve analysis of RFS in children with neuroblastoma. A Kaplan–Meier curve analysis of RFS according to the value of the NES. B Kaplan–Meier curve analysis of RFS according to the value of the LDH. C Kaplan–Meier curve analysis of RFS according to the value of 24-h VMA in urine. D Kaplan–Meier curve analysis of RFS according to the MYCN state. E Kaplan–Meier curve analysis of RFS according to the age. F Kaplan–Meier curve analysis of RFS according to the pathological type. G Kaplan–Meier curve analysis of RFS according to INSS. H Kaplan–Meier curve analysis of RFS according to the tumor capsule. I Kaplan–Meier curve analysis of RFS according to the MRD in bone marrow. NES, neuron-specific enolase; LDH, lactate dehydrogenase; VMA, vanilmandelic acid; HR, hazard ratio. NB neuroblastoma; GNBi ganglioneuroblastoma intermixed; GNBn ganglioneuroblastoma nodular
(A) NSE

P = 0.048

Increased vs Normal: HR, 5.742 (2.385-13.83)

Recurrence-free survival (months)

(B) LDH

P = 0.020

>1000 U/L vs ≤1000 U/L: HR, 1.815 (0.968-3.336)

Recurrence-free survival (months)

(C) VMA

P < 0.001

Increased vs Normal: HR, 3.012 (1.871-4.849)

Recurrence-free survival (months)

(D) MYCN state

P = 0.017

MYCN amplification vs non-MYCN amplification: HR, 1.872 (1.017-3.446)

Recurrence-free survival (months)

(E) Age

P corrected = 0.0002

≤12m, 12-18m, ≥18m

12-18m vs ≤12m: HR, 2.306 (0.587-9.060)

>18m vs 12-18m: HR, 2.096 (1.061-4.141)

Recurrence-free survival (months)

(F) Pathological type

P corrected = 0.023

GNBi, Undifferentiated or poorly differentiated NB, Differentiated NB, GNBn

Recurrence-free survival (months)

(G) INSS

P corrected < 0.0001

stage 1, 2, 4s, stage 3, stage 4

stage 3 vs stage 1, 2, 4s: HR, 4.109 (1.250-13.41)

stage 4 vs stage 3: HR, 5.275 (3.280-8.484)

Recurrence-free survival (months)

(H) Tumor capsule

P < 0.0001

Intact tumor capsule, Fractured tumor capsule

Recurrence-free survival (months)

(I) Minimal residual disease in bone marrow

P < 0.0001

Non-metastasis with bone marrow, Metastasis with bone marrow

Recurrence-free survival (months)
Results

Demographics

In this cohort, there were 131 boys (61.8%) and 81 girls (38.2%), and the male-to-female ratio was 1.61:1. Sixty-three children (29.7%) were less than 18 months old, and 249 children (70.3%) were 18 months and above. The median age at initial diagnosis was 32 months (range from 1.2 to 156.3 months). Fifty-seven cases (26.9%) were found in the physical examination without any symptoms. Forty-nine cases (23.1%) were palpated by their parents. The chief complaint of 59 cases (27.8%) was digestive system symptoms such as jaundice, vomiting, and diarrhea. The initial symptom of 21 cases (9.9%) was asthenia, muscle strength and muscle tension changes, or other motor symptoms. Cough, hemoptysis, dyspnea, and other respiratory symptoms were chief complaints in 10 patients (4.7%). Fourteen patients (6.6%) only presented with fever (6.6%). MYCN genetic testing had been carried out since 2012 in this center, so 170 out of 212 patients had been detected, including 125 (58.9%) non-amplified patients and 45 (21.2%) amplified patients. RFS rate in 1-year, 3-year, 5-year, and 10-year was 0.811, 0.662, 0.639, and 0.604, respectively. The demographic and clinical characteristics of 212 NB patients are listed in Table 1.

The primary sites of neuroblastoma were mainly retroperitoneal/paranephros (166 cases, 78.3%) and mediastinum.
Bone and bone marrow were the main metastasis sites. There were 69 patients (32.5%) with bone and bone marrow metastasis simultaneously. Eighty-nine children (42%) had bone metastases, including 10 cases (4.7%) with skull metastases, 20 cases (9.4%) with limb bone metastases, 17 cases (8%) with trunk bones metastases, and 42 cases (19.8%) with multiple bone metastases.

Eighty-two children (38.7%) had bone marrow metastasis at the initial onset. They accepted bone marrow biopsy before the therapy. The minimal residual disease (MRD) in their bone marrow was positive (MRD% ≥ 0.01%) through flow cytometers. So, they were asked to have a bone marrow puncture to monitor MRD every two cycles of chemotherapy. After two cycles, MRD turned negative (<0.01%) in 54 cases (25.5%), while MRD in 24 cases (11.3%) and 4 patients (1.9%) converted negative after four and six cycles, respectively. Univariate analysis of RFS was performed on the above three groups of patients (2, 4, and 6 cycles). The median RFS of the three groups was 25.75 months, 12.07 months, and 39.80 months, respectively (P = 0.023). Given the small number of patients whose MRD turned negative after 6 cycles of chemotherapy, the above comparison might be biased, so we compared the RFS of patients with negative MRD after 2 and 4 cycles by the Kaplan–Meier curve. (Fig. 1).

### Recurrent neuroblastoma

Seventy-one patients (33.5%) developed recurrent NB including 47 boys (66.2%) and 24 girls (33.8%). The ratio of male-to-female was 1.95:1. The median interval of recurrence from CR or VGPR was 10.27 months (range from 1.53 to 64.83 months).

### Table 3  Correlation between MYCN amplification and clinical features of neuroblastoma

| Factors         | MYCN non-amplification (No) Median (range) | MYCN amplification (No) Median (range) | P  |
|-----------------|---------------------------------------------|----------------------------------------|----|
| VMA (mg/24 h)   | 125 2.03 (0.48–35.89)                       | 45 3.29 (0.50–184.70)                  | 0.038 |
| NSE (µg/l)      | 125 74.55 (12.79–1519.00)                   | 45 244.00 (12.90–4534.00)              | 0.031 |
| LDH (U/L)       | 125 393.00 (18.50–3966.00)                  | 45 417.00 (18.50–5425.00)              | 0.07  |
| Serum ferritin  | 125 105.00 (34.90–1710.00)                  | 45 108.00 (18.60–1010.00)              | 0.665 |

LDH lactate dehydrogenase, NSE neuron-specific enolase, VMA vanillylmandelic acid

### Table 4  Multivariate analysis of RFS in recurrent neuroblastoma

| Factors         | HR 95%CI | p-value |
|-----------------|----------|---------|
| VMA             | 1.154    | 0.605–2.201 | 0.663 |
| NSE             | 1.092    | 0.136–8.795 | 0.934 |
| LDH > 1000/≤ 1000 | 1.022 | 0.535–1.954 | 0.947 |
| MYCN amplification | 1.948  | 1.075–3.530 | 0.028 |
| Bone marrow metastasis | 0.716  | 0.378–1.356 | 0.305 |
| Age(months) ≤ 12 | *       | *       | 0.037 |
|                  | 12–18    | 2.854 | 0.560–14.544 | 0.207 |
|                  | ≥ 18     | 4.682 | 1.411–15.536 | 0.012 |
| INSS 1, 2, 4 s | *       | *       | 0.002 |
|                  | 3       | 3.163 | 0.375–26.649 | 0.29  |
|                  | 4       | 16.368 | 2.075–132.286 | 0.009 |
| Histologic category | *     | *     | 0.007 |
| NB (undifferentiated or poorly undifferentiated) | 2.179 | 1.067–4.451 | 0.033 |
| NB (differentiated) | 0.325 | 0.123–0.856 | 0.023 |
| GNBi             | 2.416    | 0.493–11.845 | 0.277 |
| GNKn             | 3.313    | 1.68–6.612 | 0.001 |

The normal value of VMA: <2 mg/24 h (<2 years old), <5 mg/24 h (2–18 years old); the normal value of NSE: 0–16.3 ng/ml; the normal value of serum ferritin: 20–290 µg/l. HR denotes increased risk of the event for the second row within the given category as compared with the first row.

NB neuroblastoma, GNBi ganglioneuroblastoma intermixed, GNKn ganglioneuroblastoma nodular, MRD minimal residual disease, LDH lactate dehydrogenase, NSE neuron-specific enolase, VMA vanillylmandelic acid

*Age ≤ 12 months, INSS stage 1, 2, 4 s, and pathological type NB (undifferentiated or poorly undifferentiated) was the reference level.
### Points

**A**

| Points | 0  | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
|--------|----|----|----|----|----|----|----|----|----|----|-----|
| Age    | >18m | <12m |    |    |    |    |    |    |    |    |     |
| INSS   | 4 | Normal |    |    |    |    |    |    |    |    |     |
| VMA    | Abnormal | Normal |    |    |    |    |    |    |    |    |     |
| NSE    | Abnormal | Normal |    |    |    |    |    |    |    |    |     |
| LDH    | >1000 U/L | <1000 U/L |    |    |    |    |    |    |    |    |     |
| MYCN   | amplified | nonamplified |    |    |    |    |    |    |    |    |     |
| MRD-in.bone.marrow | negative | uqNB | GNB |    |    |    |    |    |    |    |     |
| pathological.type | GNBn | dNB | with |    |    |    |    |    |    |    |     |
| intact tumor.capsule | without |    |    |    |    |    |    |    |    |    |     |
| Total Points |    |    |    |    |    |    |    |    |    |    |     |
| Linear Predictor | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |     |
| 1-year Recurrence-Free Probability | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.8 | 0.9 |     |

**B**

| Points | 0  | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
|--------|----|----|----|----|----|----|----|----|----|----|-----|
| Age    | >18m | <12m |    |    |    |    |    |    |    |    |     |
| INSS   | 4 | Normal |    |    |    |    |    |    |    |    |     |
| VMA    | Abnormal | Normal |    |    |    |    |    |    |    |    |     |
| NSE    | Abnormal | Normal |    |    |    |    |    |    |    |    |     |
| LDH    | >1000 U/L | <1000 U/L |    |    |    |    |    |    |    |    |     |
| MYCN   | amplified | nonamplified |    |    |    |    |    |    |    |    |     |
| MRD-in.bone.marrow | negative | uqNB | GNB |    |    |    |    |    |    |    |     |
| pathological.type | GNBn | dNB | with |    |    |    |    |    |    |    |     |
| intact tumor.capsule | without |    |    |    |    |    |    |    |    |    |     |
| Total Points |    |    |    |    |    |    |    |    |    |    |     |
| Linear Predictor | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |     |
| 5-year Recurrence-Free Probability | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.7 | 0.8 | 0.9 |     |

**C**

| Points | 0  | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
|--------|----|----|----|----|----|----|----|----|----|----|-----|
| Age    | >18m | <12m |    |    |    |    |    |    |    |    |     |
| INSS   | 4 | Normal |    |    |    |    |    |    |    |    |     |
| VMA    | Abnormal | Normal |    |    |    |    |    |    |    |    |     |
| NSE    | Abnormal | Normal |    |    |    |    |    |    |    |    |     |
| LDH    | >1000 U/L | <1000 U/L |    |    |    |    |    |    |    |    |     |
| MYCN   | amplified | nonamplified |    |    |    |    |    |    |    |    |     |
| MRD-in.bone.marrow | negative | uqNB | GNB |    |    |    |    |    |    |    |     |
| pathological.type | GNBn | dNB | with |    |    |    |    |    |    |    |     |
| intact tumor.capsule | without |    |    |    |    |    |    |    |    |    |     |
| Total Points |    |    |    |    |    |    |    |    |    |    |     |
| Linear Predictor | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |     |
| 10-year Recurrence-Free Probability | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.7 | 0.8 | 0.9 |     |
while the median follow-up time was 70.80 months (range from 13.30 to 161.06 months). Forty cases (56%) relapsed within 1 year. Sixty-two cases (87.3%) were older than 18 months at the initial diagnosis. Two cases (2.8%) were in stage INSS 2; 9 cases (12.7%) were in INSS stage 3, while 60 patients (84.5%) were in INSS stage 4. Eight (11.2%) were divided into the intermediate-risk group, while 63 cases (88.8%) were divided into the high-risk group (COG). The pathological type of 40 patients was undifferentiated neuroblastoma. Forty-six (64.8%) of them had bone marrow metastases.

**Univariate analysis of RFS**

The Kaplan–Meier curve was used to conduct a univariate analysis of the correlation between clinical data and recurrent NB. The univariate analysis was performed on the relevant laboratory tests of the newly diagnosed children. Children with increased NSE values, LDH >1000 U/L, and increased VMA values had lower RFS ($P = 0.048$, $P = 0.002$, $P < 0.001$, respectively, Log-rank test (Fig. 2A, B, C). Children with MYCN amplification also had lower RFS (median: 34.03 months vs. 42.23 months, $P = 0.017$, Log-rank test, Fig. 2D). Age and RFS showed a negative correlation. The risk of recurrence was increased in older children (median: 62.30 months vs. 59.77 months vs. 36.33 months, $P$ corrected = 0.0002, Log-rank test, Fig. 2E). For the pathological subtypes, the median RFS was the longest (57.47 months) in children with GNBi and the shortest (32.60 months) in children with GNBn ($P = 0.023$, Log-rank test, Fig. 2F). RFS was correlated with tumor localization. The median RFS of stages 1, 2, and 4 s was 90.67 months; the median RFS of stages 1, 2, and 4 s was 90.67 months; and it was only 36.33 months in stage 4 ($P$ corrected < 0.0001, Log-rank test, Fig. 2G). The integrity of the tumor capsule was also correlated with RFS. Children with intact tumor capsules had a higher RFS (median: 62.60 months vs. 30.83 months, $P < 0.0001$, Log-rank test, Fig. 2H). RFS was related to whether there was bone marrow metastasis at the initial onset. Children with bone marrow metastases had lower RFS (median: 21.05 months vs. 60.28 months, $P < 0.0001$, Log-rank test, Fig. 2I). In summary, according to the univariate analysis, it can be concluded that the older onset age, with distant metastasis, bone marrow involvement, MYCN amplification, without intact tumor capsule, increased VMA and NSE value, and LDH >1000 U/L were unfavorable factors for recurrence of NB. The univariate analysis data related to RFS is shown in Table 2.

**Association between MYCN and relevant laboratory tests of NB**

The study demonstrated the correlation between the MYCN gene and the data from the relevant laboratory tests. Children without MYCN amplification had a median initial VMA value of 2.03 mg/24 h (0.48–35.89), and children with MYCN amplification had a median initial VMA value of 3.29 mg/24 h (0.50–184.70) ($P = 0.038$). The median value of initial NSE in children without MYCN amplification was 74.55 µg/l (12.79–1519.00), and the median value of initial NSE with MYCN amplification was 244.00 µg/l (12.90–4534.00) ($P = 0.031$). It demonstrated that the

| Factors | Representatives | Score |
|---------|-----------------|-------|
| Age(m) | ≤ 12 | 35 |
| | 12~18 | 20 |
| | ≥ 18 | 0 |
| INSS | Stage 1, 2, 4 s | 100 |
| | Stage 3 | 58 |
| | Stage 4 | 0 |
| VMA | Normal | 23 |
| | Increased | 0 |
| NSE | Normal | 51 |
| | Increased | 0 |
| LDH(U/L) | ≤ 1000 | 5 |
| | > 1000 | 0 |
| MYCN | Nonamplification | 29 |
| | Amplification | 0 |
| MRD in bone marrow | Negative/non-metastasis | 0 |
| Pathological type | Positive/metastasis | 5 |
| | GNBn | 0 |
| (Histologic category) | NB (undifferentiated or poorly undifferentiated) | 19 |
| | NB (differentiated) | 31 |
| | GNBi | 68 |
| | Fractured | 0 |
| Tumor capsule | Intact | 41 |

The normal value of VMA: < 2 mg/24 h (< 2 years old), < 5 mg/24 h (2–18 years old); the normal value of NSE: 0–16.3 ng/ml

NB neuroblastoma, GNBi ganglioneuroblastoma intermixed, GNBn ganglioneuroblastoma nodular, MRD minimal residual disease, LDH lactate dehydrogenase, NES neuron-specific enolase, VMA vanillylmandelic acid
MYCN gene was correlated with NSE and 24-h urine VMA value at the initial onset. The correlation between the MYCN gene and other clinical features is listed in Table 3.

**Multivariate Cox regression and nomogram**

The significant variables in the univariate analysis included age at diagnosis, tumor localization, histologic category, bone marrow metastasis, MYCN state, the integrity of tumor capsule, VMA, NSE, and LDH value. The results from multivariate Cox regression analysis of RFS showed that onset age, tumor localization, MYCN state, histologic category, and integrity of tumor capsule were independent risk factors for RFS (all \( P \) values < 0.05). The data of multivariate analysis are shown in Table 4.

Based on the multivariate analysis of RFS, all variables in the multivariate Cox regression were applied to build the nomogram model diagram for predicting RFS in the 1st year, 5th year, and 10th year, as shown in Fig. 3. Nomogram could assign points to each risk factor for recurrence, and finally, predict the RFS based on the total score individually. The corresponding meaning and description of the Arabic numbers in each risk factor in Fig. 3 are shown in Table 5. For example, if there were a 3-year-old patient with INSS Stage 4 at diagnosis, normal VMA and NES, LDH \( \leq \) 1000U/L, MYCN non-amplification, bone marrow MRD-negative, histologic category classified as undifferentiated neuroblastoma, and fracted tumor capsule, The total score was calculated as follows, \( 0 + 0 + 23 + 51 + 5 + 29 + 5 + 19 + 0 = 132 \) (scores), and the patient’s 1st-year, 5th-year, and 10th-year recurrence-free probability was approximately 87%, 62%, and 45% referring to Fig. 3, respectively.

Two methods were taken to verify the model:

1. Used the Bootstrap method and repeated the sample 1000 times for internal verification. It showed that the recurrence prediction model had a C-index of 0.824 (\( P = 0.023 \)).
2. Used the graph calibration method to verify the RFS of the 1st-year, 5th-year, and 10th-year RFS. See Fig. 4 for details. It showed that the prediction curve of 1-year, 5-year, and 10-year was entangled with the standard curve, indicating that the model had good predictive potential.

**Discussion**

Neuroblastoma was one of the common extracranial solid tumors in childhood. The proportion of boys was larger in the analysis of clinical characteristics. However, gender
was not the factor affecting RFS. It had been reported that boys had a higher incidence than girls, but the reason was not yet apparent, and further research was needed [2]. The relationship between onset age and time to recurrence was still unclear. This study concluded that onset age was an independent factor affecting RFS. The older children would be prone to earlier relapse. The prognosis of recurrent neuroblastoma was poor. Garaventa et al. [22] reported that the 10-year overall survival (OS) rate of relapsed patients was 14.4%, and age over 18 months was a poor prognostic factor. Most of the 71 relapsed cases in this cohort were 18 months and older, INSS stage 4, with bone marrow metastases. Therefore, the recurrence should be paid more attention to the first-onset patients with the clinical characteristics above.

More than half of children with neuroblastoma were accompanied by distant metastases at the first diagnosis, especially bone and bone marrow metastasis. It was consistent with the data of this study. According to Fig. 1, the later the MRD of bone marrow turned negative, the earlier the children would relapse. The reason might be that if tumor cells in the bone marrow were more sensitive to chemotherapy, the tumor cells in the primary site were also sensitive to chemotherapy, and the treatment effect was better, and vice versa. It suggested that if patients developed bone marrow metastasis and positive MRD for a long time, it was necessary to increase the frequency of follow-up and shorten the time interval between follow-ups.

MYCN gene amplification was an essential prognostic factor for neuroblastoma. The 3-year OS of children with MYCN amplification was less than 30%. It had been reported that MYCN amplification was related to the primary abdomen, high LDH, massive tumor, poor pathological types, and chromosome aberration [21]. This study showed that 21.2% of children presented MYCN amplification, and the MYCN state was also an independent influencing factor for RFS. Children with MYCN amplification relapsed earlier, and the reason might be that the MYCN amplification was positively related to tumor aggressiveness.

INPC proposed a prognostic classification based on age and histologic category, which was divided into favorable histology (FH) and unfavorable histology (UH) [16]. GNBi of any age was classified as FH group, and GNBn of any age was in the UH group. Nakazawa et al. [22] verified that the prognosis of the FH group was better than the UH group (OS rates were 88.6% and 43.1%, respectively). Sokol et al. [23] studied the influence of histologic category in INPC on the prognosis. The 5-year EFS of GNBi was greater than 85%, and the 5-year EFS of NB (differentiated) was higher than that of NB (undifferentiated or poorly undifferentiated). This study concluded that histologic category could be an independent factor for RFS. The RFS of GNBi was the longest, while the RFS of GNBn was the shortest, which was consistent with the trend of the above-mentioned prognostic analysis.

The tumor localization and tumor capsule were independent prognostic factors that affect RFS. Children with localized tumors and intact capsules had longer RFS. The reason might be that surgeons could completely remove those localized tumors with intact capsules. Li et al. [24] also discussed that pediatric neuroblastomas in the head and neck were easier to complete resection if bulks with clear borders and intact capsules. So, these children’s risks of recurrence were reduced.

Other researchers had reported that the VMA value was correlated with MYCN amplification [20, 25, 26]. Cangemi et al. [27] found that the ratio of urine VMA/HVA was an independent predictor of prognosis in patients with localized NB and MYCN non-amplification. LDH had an independent prognostic value in patients at all stages without MYCN amplification. In this study, MYCN amplification was correlated with the NSE value at the initial onset and 24-h urine VMA value. It also showed that NSE value and 24-h urine VMA value were significant predictors of recurrence in univariate analysis. Now, in China, few centers could perform MYCN state testing for pediatric NB. Doctors might indirectly speculate about the MYCN state through these laboratory tests for children who had not been tested for MYCN state for any reason. If their NSE and 24-h urine VMA values were abnormal, we could indicate that the MYCN gene amplification was likely to be positive, and the risk of recurrence was correspondingly increased. Therefore, these patients should be paid more attention to follow-ups after CR or VGPR.

Researchers and members from major countries and international cooperative organizations in North America, Europe, and Asia had developed the International Neuroblastoma Risk Group (INRG) Classification and Staging System (INRGS), which used preoperative radiological features and image-defined the risks factors (IDRFs) to distinguish low-risk tumors from high-risk tumors [28, 29]. It analyzed 13 clinical and biological variables on the impact of event-free survival, including two variables, LDH and serum ferritin [30]. This study also incorporated the two indicators and found that LDH value of more than 1000 U/L was an important factor for RFS, while serum ferritin had no statistically significant effect on RFS \((P = 0.082)\). It might be due to the poor specificity of serum ferritin for NB. The laboratory tests concerning VMA, NES, and LDH had a critical influence on RFS in univariate analysis, in which the values would be tested at the initial onset on most Chinese NB patients. Further comparison of the three showed that the hazard ratio (HR) value was the highest in the NSE group (3.012 vs 5.742 vs 1.815) (see Fig. 2A, B, and C for details). It had been reported that more than 90% of NB children had an abnormally increased NSE value on the first onset [31]. Furthermore, Zelter [32] reported that NSE showed a significant correlation with INSS; patients with metastases had higher NSE.
values. So, NSE value was of much more value for the early diagnosis and recurrence. For children with abnormal NSE values, we should shorten the follow-up interval, increase the frequency of follow-up, and closely monitor their recurrence. The nomogram model was accurate and concise. It could display the results graphically. It was widely used in clinical model prediction. Liang et al. [33] incorporated eight prognostic factors to build a nomogram model to predict the OS rate of NB based on the SEER database. The applicability needed further verification for Chinese patients because nomogram clinical prediction model was based on public databases, not Chinese patients; besides, they did not target the recurrent NB patients. This research had certain limitations. The dataset was limited to data from a single center and of small size, which might be less convincing to demonstrate the effectiveness and robustness of the proposed method. We conducted internal verification on the nomogram, but further external verification should have been determined whether it could be universally applied. Indeed, this needed to be validated in a larger and external “data validating” group.

Conclusion

Increased VMA and NSE value and LDH > 1000 U/L were important adverse factors for the recurrence of NB. NSE value was more valuable for predicting NB recurrence. Age at diagnosis, tumor localization, MYCN state, histologic category, and tumor capsule were independent risk factors for the recurrence of NB. The results demonstrated that the nomogram could accurately predict the recurrence of children with neuroblastoma.

Acknowledgements The authors wish to thank the patients and the staff in the Department of Pediatric Hematology/Oncology, Xinhua Hospital Affiliated to Medicine School of Shanghai Jiaotong University.

Authors' contributions All the authors contributed to the study conception and design. Data collection, follow-up, and analysis were performed by Quan Sun, Yanmin Chen, Qianya Jin, and Xiaojun Yuan. The first draft of the manuscript was written by Quan Sun, and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

Declarations

Ethics approval The ethics approval was granted by the ethics committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Conflict of interest The authors declare no competing interests.

References

1. Tsubota S, Kadomatsu K (2018) Origin and initiation mechanisms of neuroblastoma. Cell Tissue Res 372(2):211–221
2. Whittle SB, Smith V, Doherty E, Zhao S, McCarty S, Zage PE (2017) Overview and recent advances in the treatment of neuroblastoma. Expert Rev Anticancer Ther 17(4):369–386
3. Maris JM (2010) Recent advances in neuroblastoma. N Engl J Med 362(23):2202–2211
4. Swift CC, Eklund MJ, Kraveka JM, Alazraki AL (2018) Updates in diagnosis, management, and treatment of neuroblastoma. Radiographics 38(2):566–580
5. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A (2014) Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin 64(2):83–103
6. Olsen HE, Campbell K, Bagatell R, DuBois SG (2020) Trends in conditional survival and predictors of late death in neuroblastoma. Pediatr Blood Cancer 67(10):e28329
7. London WB, Castel V, Monclair T, Ambros PF, Pearson AD, Cohn SL et al. (2011) Clinical and biologic features predictive of survival after relapse of neuroblastoma: a report from the International Neuroblastoma Risk Group project. J Clin Oncol 29(24):3286–3292
8. Brodeur GM, Pritchard J, Berthold F, Carl森 NL, Castel V, Castelberry RP et al (1993) Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol 11(8):1466–1477
9. Kushner BH, Kramer K, Modak S, Cheung NK (2009) Sensitivity of surveillance studies for detecting asymptomatic and unsuspected relapse of high-risk neuroblastoma. J Clin Oncol 27(7):1041–1046
10. Su Y, Ma XL, Wang HM, Qin H, Qin MQ, Zhang FQ et al (2020) Clinical characteristics and prognostic analysis of 458 children with high-risk neuroblastoma in a single center. Zhonghua Er Ke Za Zhi 58(10):796–801
11. Chang HH, Lu MY, Yang YL, Chou SW, Lin DT, Lin KH et al (2020) The prognostic roles of and correlation between ALK and MYCN protein expression in neuroblastoma. J Clin Pathol 73(3):154–161
12. Iasonos A, Schrag D, Raj GV, Panageas KS (2008) How to build and interpret a nomogram for cancer prognosis. J Clin Oncol 26(8):1364–1370
13. Balachandran VP, Gonen M, Smith JJ, DeMatteo RP (2015) Nomograms in oncology: more than meets the eye. Lancet Oncol 16(4):e173–e180
14. Kim SY, Yoon MJ, Park YI, Kim MJ, Nam BH, Park SR (2018) Nomograms predicting survival of patients with unresectable or metastatic gastric cancer who receive combination cytotoxic chemotherapy as first-line treatment. Gastric Cancer 21(3):453–463
15. Wang J, Yang B, Li Z, Qu J, Liu J, Song N et al. (2020) Nomogram-based prediction of survival in unresectable or metastatic gastric cancer patients with good performance status who received first-line chemotherapy. Ann Trans Med 8(6):311
16. Shimada H, Ambros IM, Dehner LP, Hata J, Joshi VV, Roald B et al (1999) The International Neuroblastoma Pathology Classification (the Shimada system). Cancer 86(2):364–372
17. Peuchmaur M, d’Amore ES, Joshi VV, Hata J, Roald B, Dehner LP et al. (2003) Revision of the International Neuroblastoma Pathology Classification: confirmation of favorable and unfavorable prognostic subsets in ganglioneuroblastoma, nodular. Cancer 98(10):2274–2281
18. Wang Y, Wu Y, Wang L, Yuan X, Jiang M, Li Y (2017) Analysis of Recurrent sacrococcygeal teratoma in children: clinical
features, relapse risks, and anorectal functional sequelae. Med Sci Monit 23:17–23

19. Wu ZY, Shen W, Yue JQ, Yao WY, Liu SL, Jin YP et al (2020) Combining immunoscore with clinicopathologic features in cholangiocarcinoma: an influential prognostic nomogram. Onco Targets Ther 13:11359–11376

20. Garaventa A, Parodi S, De Bernardi B, Dau D, Manzitti C, Conte M et al (2009) Outcome of children with neuroblastoma after progression or relapse. A retrospective study of the Italian neuroblastoma registry. Eur J Cancer 45(16):2835–42

21. Lee JW, Son MH, Cho HW, Ma YE, Yoo KH, Sung KW et al (2018) Clinical significance of MYCN amplification in patients with high-risk neuroblastoma. Pediatr Blood Cancer 65(10):e27257

22. Nakazawa A, Haga C, Ohira M, Okita H, Kamijo T, Nakagawara A (2015) Correlation between the International Neuroblastoma Pathology Classification and genomic signature in neuroblastoma. Cancer Sci 106(6):766–771

23. Sokol E, Desai AV, Applebaum MA, Valteau-Couanet D, Park JR, Pearson ADJ et al (2020) Age, diagnostic category, tumor grade, and Mitosis-Karyorrhexis Index are independently prognostic in neuroblastoma: an INRG Project. J Clin Oncol 38(17):1906–1918

24. Li YZ, Liu YW, Wang SC, Tai J, Zhang J, Liu YH et al (2019) Clinical analysis of head and neck neurogenic tumor in childhood. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 33(10):983–986

25. Parodi S, Papio F, Haupt R, Conte M, De Bernardi B (2007) The prognostic role of urinary catecholamines in infants with disseminated neuroblastoma might be mediated by MYCN amplification. Pediatr Blood Cancer 48(5):593; author reply -4

26. Strenger V, Kerbl R, Dornbusch HJ, Ladenstein R, Ambros PF, Ambros IM et al (2007) Diagnostic and prognostic impact of urinary catecholamines in neuroblastoma patients. Pediatr Blood Cancer 48(5):504–509

27. Cangemi G, Reggiardo G, Barco S, Barbagallo L, Conte M, D’Angelo P et al (2012) Prognostic value of ferritin, neuron-specific enolase, lactate dehydrogenase, and urinary and plasmatic catecholamine metabolites in children with neuroblastoma. Onco Targets Ther 5:417–423

28. Monclair T, Brodeur GM, Ambros PF, Briste HJ, Cecchetto G, Holmes K et al (2009) The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. J Clin Oncol 27(2):298–303

29. Briste HJ, McCarville MB, Granata C, Krug KB, Wootton-Gorges SL, Kanegawa K et al (2011) Guidelines for imaging and staging of neuroblastic tumors: consensus report from the International Neuroblastoma Risk Group Project. Radiology 261(1):243–257

30. Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, Brodeur GM et al (2009) The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. J Clin Oncol 27(2):289–297

31. Simon T, Hero B, Hunneman DH, Berthold F (2003) Tumour markers are poor predictors for relapse or progression in neuroblasts. Eur J Cancer 39(13):1899–1903

32. Zeltzer PM, Maragos PJ, Parma AM, Sather H, Dalton A, Hammond D et al (1983) Raised neuron-specific enolase in serum of children with metastatic neuroblastoma. A report from the Children’s Cancer Study Group. Lancet 2(8346):361–3

33. Liang SW, Chen G, Luo YG, Chen P, Gu JH, Xu QQ et al (2020) Nomogram for predicting overall survival in children with neuroblastoma based on SEER database. Ann Surg Treat Res 99(2):118–126

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.