Prediction to the prognosis of children with neuroblastoma by nomogram based on the first-diagnosed inflammatory markers

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Research Article

Keywords:

Posted Date: September 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2100392/v1

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Abstract

**Background:** Patients with high-risk neuroblastoma (NB) have a poor prognosis. The prognostic significance of inflammatory biomarker-based nomograms for children with NB has not been previously studied.

**Methods:** Part of patients diagnosed with NB in our center from January 2016 to March 2022 were included in the study. Inflammatory biomarkers were primary outcome measures, including C-reactive protein (CRP), ferritin, neutrophil to lymphocyte ratio (NLR), and lymphocyte to monocyte ratio (LMR), platelet to lymphocyte ratio (PLR) and systemic immune-inflammation index (SII). Univariate and multivariate survival analyses were performed to assess the prognostic value of these indicators for overall survival (OS) in NB children, showing the Kaplan-Meier survival curves and plotting the nomogram. C-index were used to detect predictability.

**Results:** 93 NB patients were retrospectively analyzed. CRP, ferritin, NLR, PLR, and SII were significantly associated with OS of NB patients, while LMR were found to be not predictive of OS for NB patients. The established nomogram is well-calibrated, and the C-index is 0.731.

**Conclusion:** Survival analysis found part of inflammatory biomarkers related to the prognosis of NB. The nomogram could be used as a convenient predictive tool in clinical practice to evaluate the prognosis of NB children at first diagnosis.

**Impact**

1. CRP, ferritin, NLR, PLR, and SII are associated with the prognosis of neuroblastoma.

2. We have created a nomogram that can be used to predict the prognosis of affected children and play a role in determining the treatment plan.

3. Our results differ from a previous study in that we increased the sample size for the study and had good internal validation results for the nomogram.

1. **Introduction**

Neuroblastoma is a neuroendocrine tumor that arises in the developing sympathetic nervous system (from any neural crest element), which results in tumors in the adrenal glands and/or sympathetic ganglia\(^1\). 90% of tumors arise in children who are <10 years of age, and neuroblastoma has a median age at diagnosis of 18 months\(^2\). For children with high-risk metastatic and/or relapsed neuroblastoma, survival remains poor and long-term morbidities are common. With the integration of anti-disialoganglioside (GD)2 monoclonal antibody (mAb) into the standard of therapy, 50%-60% of children with high-risk neuroblastoma (HR-NB) are long-term survivors\(^3\).
Inflammation represents the link between internal factors that contribute to tumor development, including oncogenes, tumor suppressors, and genomic stability genes, and external factors, including immune and matrix components\[4\], and it has become a new direction of tumor therapy, namely immunotherapy. Almost 20% of human cancers are related to chronic inflammation caused by infections, exposure to irritants or autoimmune disease\[5\], including infection with H. pylori and hepatitis B. In recent years, several blood inflammation biomarkers or inflammation-based scores have been found to predict the survival of many types of cancer, including C-reactive protein (CRP), albumin (ALB), neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR), platelet to lymphocyte ratio (PLR) and systemic immune-inflammation index (SII), and these indicators are associated with tumor prognoses such as hepatocellular carcinoma (HCC)\[6\], colorectal cancer (CRC) \[7\], endometrial carcinoma\[8\] and cervical cancer\[9\].

At present, there are relatively comprehensive risk assessment standards according to age, pathological typing, MYCN amplification, 11q deletion, and other indicators, which can guide the prognosis of clinical work. However, there is a lack of a simple and convenient predictive tool for the overall disease prognosis evaluation system to determine the prognosis of the child at the time of initial diagnosis. A previous study\[10\] has demonstrated the prognostic value of inflammatory biomarkers including C-reactive protein (CRP), albumin (ALB), Glasgow Prognostic Score (GPS) and C-reactive protein to albumin ratio (CAR) among other inflammatory biomarkers for the prognostic value of survival in NB patients, but with the preliminary analysis of the data of our center, there are some differences. Therefore, we conducted this retrospective study in which we expanded the sample size, introduced new indicators to analyze the prognosis of children with NB, and developed a predictive model through a nomogram to complement the existing staging risk system and assist in clinical work.

2. Methods

2.1 Study population

This study retrospectively analyzed the database of the Department of Surgical Oncology, Children's Hospital of Chongqing Medical University from January 2016 to March 2022. The inclusion criteria were listed below: (1) patients pathologically diagnosed with ganglioneuroma (GN), ganglioneuroblastoma (GNB), or neuroblastoma (NB); (2) patients with complete follow-up information and clear endpoints. Patients with the following criteria were excluded: (1) signs of infectious disease suggested by the admission examination; (2) patients with incomplete survival information. A total of 101 cases were collected, and 93 cases were eventually enrolled. All eight children had varying degrees of cough and sputum, along with signs of fever, and were clinically diagnosed with acute upper respiratory tract infection or bronchopneumonia.

67 patients were treated with chemotherapy according to the course of treatment after radical resection of tumor and 26 patients received neoadjuvant chemotherapy followed by radical surgery and conventional chemotherapy.
All children underwent tumor resection or biopsy with definite pathological results. The patient’s last contact data was used as the end of the follow-up. All patients who died were followed up with the patient's last contact data as the end of follow-up. The use of clinical data was reviewed and approved by the Institutional Review Board of Chongqing Medical University, Chongqing, China.

2.2 Data collection

The collected clinical and pathological data in this study were listed as follows: age, the Shimada histologic classification system, hematological indexes of initial diagnosis (NLR, PLR, LMR, and SII), ferritin, C-reactive protein (CRP), NMYC amplification or not, tumor size, and INSS (International Neuroblastoma Staging System) stage. NLR is the ratio of neutrophil count to lymphocyte count. PLR is the ratio of platelet count to lymphocyte count. LMR is the ratio of lymphocyte count to monocyte count. SII is the platelet count x neutrophil count/lymphocyte count [6, 7, 10-12].

2.3 Statistical analysis

Data were analyzed using the SPSS Statistics software version 26 (IBM Corporation, USA) the software GraphPad Prism 8 and R version 4.1.3 software (The R Foundation for Statistical Computing, Vienna, Austria.) Receiver operating characteristic (ROC) curve analysis was performed to analyze the area under the ROC curve (AUC), and the Youden Index (sensitivity + specificity – 1) was used to identify the optimal cut-off values for each continuous variable. Survival analysis was performed using the Kaplan-Meier method and logarithmic rank test to compare survival differences. Univariate multivariate analysis was conducted by Cox proportional risk regression model. The results were presented as hazard ratios (HRs) and 95% confidence intervals (CI). \( P \lt 0.05 \) was considered statistically significant.

Variables with statistically significant differences in the multivariate Cox proportional hazard model (log-rank test, \( P \lt 0.05 \)) were chosen to build the nomograms. The performance of the nomogram was measured by concordance index [C-index, equivalent to the area under the receiver operating characteristic curve (AUROC)]. The R packages “survival”, “rms”, “Hmisc”, “lattice”, “Formula”, “ggplot2”, and “splines” were applied.

3. Results

3.1 Patient characteristics

A total of 93 patients were included in this study. The age of 59 patients at the time of diagnosis was greater than or equal to 18 months, and the other 34 patients were less than 18 months. The tumor size was less than 5 cm in 20 cases and more than 5 cm in 73 cases. The end-point follow-up time of dead patients was the time of death. After a definite pathological diagnosis, there were 73 cases of NB and 20 cases of GNB.

3.2 ROC analysis
ROC analysis (Figure 1) was performed and the Youden Index was calculated to determine the best cut-off point with the highest sensitivity and specificity. Ferritin was 123.5, NLR was 0.84, PLR was 144.1, and SII was 488.9, while the results of LMR were not satisfactory: $P=0.065$. Thus LMR would not be considered in the following analysis.

According to the sensitivity and specificity (Figure 1), for each index, patients were divided into two groups for further analysis: [Ferritin $\leq$ 123.5 (low) and Ferritin $\geq$ 123.5 (high) NLR $\leq$ 0.84 (low) and NLR $\geq$ 0.84 (high); PLR $\leq$ 144.1 (low) and PLR $\geq$ 144.1 (high); SII $\leq$ 488.9 (low) and SII $\geq$ 488.9 (high)]. Due to the inspection standard of our hospital, we regard CRP $\geq$ 8 as the elevated group and CRP < 8 as the normal group.

### 3.3 Survival Analysis for Overall Neuroblastoma Patients

The mean follow-up time for this study was 54.2 months. In this cohort, the median OS was 33 months, while the 1, 3, and 5-years overall survival rate was 87.10%, 66.67%, and 64.52%, In addition to these inflammation biomarkers, clinical data such as the age of diagnosis, tumor size, and INSS stage were also included in the analysis (Figure 2 A-J).

The median survival time of patients in the high NLR, PLR, and SII group was 29.5, 27.5, and 29 months, and in the low NLR, PLR, and SII group was 40, 36, and 37 months respectively. Using the log-rank test, the low NLR, PLR, and SII group statistically had better long-term overall survival outcome than the high NLR, PLR, and SII group respectively (Figure 2 G, H, I).

The OS of patients with elevated CRP levels was significantly lower than that of patients with normal CRP levels (Figure 2A, $P=0.001$). Patients with higher NLR, PLR, SII, and ferritin values had significantly worse OS than those with lower NLR (Figure 2G, $P=0.003$), PLR (Figure 2H, $P=0.001$), SII (Figure 2I, $P=0.002$) and ferritin (Figure 2D, $P=0.001$) value.

Univariate survival analysis showed that Age (HR=4.271; 95% CI=1.638-11.137; $p=0.003$), INSS stage (HR=7.363; 95% CI=1.760-30.796; $p=0.006$), Tumor size (HR=4.876; 95% CI=1.165-20.402; $p=0.03$), Shimada (HR=17.681; 95% CI=4.166-75.048; $p=0.001$), N-MYC (HR=4.664; 95% CI=2.224-9.780; $p=0.001$), CRP (HR=5.902; 95% CI=2.839-12.269; $p=0.001$), Ferritin (HR=11.223; 95% CI=4.137-30.449; $p=0.001$), NLR (HR=3.783; 95% CI=1.458-9.817; $p=0.006$), PLR (HR=3.435; 95% CI=1.702-6.931; $p=0.001$) and SII (HR=2.871; 95% CI=1.425-5.783; $p=0.003$) were significantly associated with OS of the patients.

Then, multivariate survival analysis was performed on the all variables above, the results showed that ferritin (HR=7.184; 95% CI=2.005-25.748; $p=0.002$) and Shimada (HR=32.868; 95% CI=2.907-371.653; $p=0.005$) was significantly associated with OS of NB patients (Table 1). Next, we took the seven indicators of size, age, CRP, ferritin, NLR, PLR and SII as a group for multivariate analysis, which can be obtained at the initial diagnosis. We found that only ferritin (HR =8.532; 95% CI=2.412-30.179; $p=0.001$) was significantly associated with OS of NB patients.
Table 1

Univariate and Multivariate Cox Regression Analyses for OS of the patients with NB
<table>
<thead>
<tr>
<th>Variables</th>
<th>Total No. (Death No.)</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HR(95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Age</td>
<td>≥18 months</td>
<td>4.271(1.638-11.137)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>18 months</td>
<td></td>
<td>/</td>
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<tr>
<td>INSS stage</td>
<td>I and II</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>III and</td>
<td>66 31</td>
<td>/</td>
</tr>
<tr>
<td>Tumor size</td>
<td>≥5cm</td>
<td>4.876(1.165-20.402)</td>
<td>0.03</td>
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<tr>
<td></td>
<td>5cm</td>
<td>20 2</td>
<td>/</td>
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<tr>
<td>Shimada</td>
<td>FH</td>
<td>39 (2)</td>
<td>/</td>
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<tr>
<td></td>
<td>uFH</td>
<td>50 (29)</td>
<td>17.681 4.166-75.048</td>
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<td>N-MYC</td>
<td>amplification</td>
<td>4.664 2.224-9.780</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>non-amplified</td>
<td>75 (19)</td>
<td>/</td>
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<tr>
<td>CRP</td>
<td>≥8 mg/L</td>
<td>5.902(2.839-12.269)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>8 mg/L</td>
<td>66 12</td>
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<tr>
<td>Ferritin</td>
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<td>11.223(4.137-30.449)</td>
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<td></td>
<td>123.5ng/ml</td>
<td>44 6</td>
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<tr>
<td>NLR</td>
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<td>0.84</td>
<td>35 5</td>
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<tr>
<td>PLR</td>
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<td>3.435(1.702-6.931)</td>
<td>0.001</td>
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<tr>
<td></td>
<td>144.1</td>
<td>61 13</td>
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<tr>
<td>SII</td>
<td>≥488.9</td>
<td>2.871(1.425-5.783)</td>
<td>0.003</td>
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<td></td>
<td>488.9</td>
<td>58 13</td>
<td>/</td>
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<td>Variables</td>
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<td></td>
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<td>HR(95% CI)</td>
<td>P value</td>
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<tr>
<td>Tumor size</td>
<td>≥5cm 5cm</td>
<td></td>
<td></td>
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<tr>
<td>Age</td>
<td>≥18 months 18 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>≥8 mg/L 8 mg/L</td>
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</tr>
<tr>
<td>Ferritin</td>
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<td>8.532 2.412-30.179</td>
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<td>NLR</td>
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<tr>
<td>PLR</td>
<td>≥144.1 144.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SII</td>
<td>≥488.9 488.9</td>
<td></td>
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</tbody>
</table>

**Abbreviations**: HR, hazard ratio; CI, confidence interval; INSS, International Neuroblastoma Staging System; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SII, system inflammation index; CRP, C-reactive protein.

3.4 **Construction of the nomogram for 3-5-year OS**

Based on the results of COX regression analyses, all the predictors of OS in the whole study were integrated into the nomograms. Figure 3 illustrates the predictive nomograms for the 3-5-year OS. By adding the score of each selected variable, the individual survival probability of the patient can be easily calculated. Each variable is projected upward to the value of the small scale (point) to obtain the score of each parameter. The lower the score, the worse the prognosis. The nomograms demonstrated good predictive performance for OS, with a C-index of 0.731. Because the expected nomogram was predicted based on the time point of the first diagnosis and the determination of INSS stage, Shimada, and NMYC amplification or not usually required pathological results, the nomogram was not included in these indicators. Under the guidance of a nomogram, we could judge the prognosis of patients at the first hospitalization according to the different characteristics of each patient.
In the prediction model, for the variable “Age”, “1” meant the patient was 18 months or older at the time of diagnosis; for the variable “CRP”, “1” meant the patient’s serum CRP level was greater than or equal to 8mg/L; for the variable “size”, “1” meant the patient’s tumor diameter was greater than or equal to 5cm. For the four metrics, Ferritin, NLR, PLR and SII, the scores can be calculated by matching the specific values to the scale. By simple numerical addition, we can predict the three-year and five-year survival probability of patients with neuroblastoma.

As shown in Figure 4, the calibration curves indicated good agreement between the predicted and observed probabilities.

4. Discussion

Pediatric solid tumors are considered to possess low immunogenicity, but numerous epidemiological studies have also demonstrated the importance of immune cells in NB\textsuperscript{[13-15]}, including potential impacts on metastasis and prognosis. Therefore, those various inflammatory mediators produced by inflammatory cells also have an important role in the development of tumors. Our results suggest that ferritin, NLR, PLR, and SII values at initial diagnosis were all predictive of survival outcomes in our cohort, and that ferritin was the only independent prognostic factor. Compared with the established tumor staging and risk classification system, these indicators are simple, easy to obtain, and convenient. SII and other indicators are newly proposed in recent years, but they have shown extraordinary ability to predict prognosis in various human solid tumors. Nomograms created with data from retrospective studies to predict the prognosis of children with neuroblastoma have not been retrieved.

Ferritin, as one of the tumor markers, is generally considered to be related to the poor prognosis of children with neuroblastoma. This is consistent with the multivariate analysis. A previous ferritin study of a large sample of neuroblastoma patients found that ferritin had a strong impact on the prognosis of high-risk NB patients receiving modern treatment after 2009, and ferritin show promise for (1) identifying ultra-high-risk; (2) refining risk stratification; and (3) clinical utility in low-/middle-income countries\textsuperscript{[16]}. In addition, ferritin plays an important role in peptide-based active delivery of nanomedicines to neuroblastoma cells\textsuperscript{[17]}. This is consistent with the findings of our study.

Inflammatory molecules, including cytokines and chemokines, are dysregulated in many tumor types, including neuroblastoma\textsuperscript{[18, 19]}. We find that NLR, PLR, and SII play an important role in predicting the survival of NB patients. This is consistent with the results of various solid tumors reported so far, such as HCC and CRC. But our results are different from previous studies\textsuperscript{[10]}; we believe that indicators including NLR, PLR, and SII are associated with the prognosis of children with neuroblastoma, so more samples are needed for retrospective analysis and prospective research in the future.

The expression of inflammation-related CD16, CD33, IL-6R, IL-10, and FCGR3 in patients with neuroblastoma at ≥ 18 months was higher than that in patients diagnosed at < 18 months\textsuperscript{[20]}. The transplanted mouse model of neuroblastoma cells confirmed that the overexpression of IL-6 was related
to the increase in tumor growth rate\textsuperscript{[21]}. Tumor-associated macrophages (TAMs) can promote neuroblastoma growth, metastasis, and the development of drug resistance\textsuperscript{[20]}, and TAMs represent a negative prognostic factor for neuroblastoma\textsuperscript{[22]}. The increased expression of CXCR4 in chemokines is associated with advanced clinical stage and the presence of bone marrow metastasis\textsuperscript{[23]}, hypoxia-induced transcription factor-1 (HIF-1\textalpha{}, HIF-2\textalpha{}) development, and metastasis of drug resistance\textsuperscript{[24]}.

There is no doubt that these results have a sound theoretical basis. As a non-specific serum biomarker of inflammatory response in the acute phase, CRP has shown its predictive role in patients with advanced non-small cell lung cancer (NSCLC) receiving anti-PD-1 immunotherapy: elevated pre-treatment CRP levels were an independent predictor of worse PFS, while the trajectory of elevated CRP during anti-PD-(L)1 therapy was a strong predictor of elevated risk of progression\textsuperscript{[25]}. This is undoubtedly instructive for NB. Neutrophils can mediate cytotoxicity and growth inhibition via chimeric anti-GD2 antibodies, but can also promote tumor cell growth if antibodies are not present or GD2 is not expressed\textsuperscript{[26]}. A type of lymphocyte, the Tumor-infiltrating lymphocytes(TIL), play an essential role in improving the clinical outcome of neuroblastoma (NB) patients\textsuperscript{[27]}.

Our study also has several limitations. Compared to the previous study, the proportion of stage III/patients in the data is higher, this may lead to bias in the experimental conclusion, but the C-index of nomograms is satisfactory. Besides, about a dozen patients had incomplete ferritin data, which reminds us to improve screening for tumor markers after admission for all patients with suspected tumors. If we can refine the relevant tests including humoral and cellular immunity, or interleukin and calcitoninogen, the results may be richer.

**5. Conclusion**

In this retrospective study, We find that CRP, ferritin, PLR, NLR, and SII are all associated with the prognosis of patients with NB, we further built the nomograms to estimate 3 5-year OS, and This personalized predictive tool will help supplement existing staging and risk classification systems, perform prognostic assessments, help guide subsequent treatment, and potentially assist in determining treatment intensity based on predicted outcomes.

**Declarations**

**Funding**

This work was supported in part by research grants from the Key Project of “Research on Prevention and Control of Major Chronic Non-Communicable Diseases”, the Ministry of Science and Technology of the People’s Republic of China, National Key R&D Program of China (No. 2018YFC1313000, 2018YFC1313004), the General Clinical Medical Research Program of Children's Hospital of Chongqing Medical University (No. YBXM-2019-003).
Ethics approval

The clinical sample study protocol was reviewed and approved by the Institutional Review Board of the Children's Hospital of Chongqing Medical University.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Patient consent

This study is a retrospective study that has been approved by the ethics committee and does not require patient consent.

Author contributions

S. W. was responsible for the general conception of the experiment as well as the planning, and for revising the manuscript. Y.L.Z. collected the data, performed most of the experiments and data analysis, and wrote the manuscript. C.H.Z. and Y.M. collected the data and performed a partial data analysis. C.C.L., Z.Z.Z., J.W.Z., L.P., and X.B.D. contributed to the sample collection. All authors reviewed, edited and approved the manuscript.

Data Availability Statement

The datasets generated and analyzed in this study are not publicly available due to the patient privacy implications of some of the data, but are available from the corresponding authors upon reasonable request.

References


**Figures**

![ROC curves for the prognostic role of the inflammation biomarkers in 93 patients with NB.](image)

**Figure 1**

The ROC curves for the prognostic role of the inflammation biomarkers in 93 patients with NB.

**Abbreviations:** AUC, area under curve; CI, confidence interval; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SII, system inflammation index;
Figure 2

Kaplan–Meier survival curves for OS in a total of 93 patients with NB according to (A) CRP, (B) INSS, (C) Shimada, (D) Ferritin, (E) N-MYC, (F) Age, (G) NLR, (H) PLR, (I) SII, (J) size.

Abbreviations: INSS, International Neuroblastoma Staging System; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SII, system inflammation index; CRP, C-reactive protein.
Figure 3

Nomogram predicting 3 5-year OS of patients with neuroblastoma

**Abbreviations:** overall survival
Figure 4

Calibration of the nomogram.