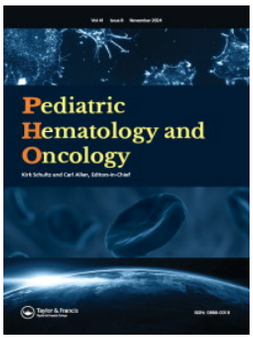

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The relationship between circulating tumor cells in peripheral blood and clinical characteristics of pediatric neuroblastoma and prognostic evaluation

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ABSTRACT

This study investigates the correlation between circulating tumor cells (CTCs) in peripheral blood and the clinical characteristics and prognosis of advanced pediatric neuroblastoma (NB). We conducted a retrospective analysis of 144 children with advanced NB who underwent comprehensive treatment. Detailed clinical data were collected, and CTCs were detected using a negative enrichment method combined with immunofluorescence technology. Prognostic evaluation criteria and cutoff values for CTCs were established using ROC curve analysis. Univariate and Cox multivariate regression analyses identified independent risk factors impacting prognosis. Patients were categorized into high and low-expression groups based on optimal cutoff values determined with X-tile software. The high expression group had a significantly higher incidence of disease progression ($p<0.001$), maximum tumor diameter ≥10 cm ($p=0.004$), undifferentiated subtype ($p=0.034$), and stage IV disease ($p=0.007$) compared to the low expression group. CTCs were notably higher in patients with progression compared to those with mitigation ($p<0.001$), in those with maximum tumor diameter ≥10 cm compared to <10 cm ($p<0.001$), and in stage IV compared to stage III patients ($p=0.036$). The AUC values for maximum tumor diameter, degree of differentiation, and tumor stage were 0.703, 0.669, 0.574, and 0.598, respectively. The detection of CTCs provides significant insights into the clinical characteristics and prognosis of advanced pediatric NB, highlighting its potential as a prognostic tool.

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Circulating tumor cells; comprehensive treatment; immunomagnetic bead adsorption; neuroblastoma; prognostic evaluation

Introduction

Neuroblastoma (NB) is the most common extracranial solid tumor in infants and young children, ranking as the third most common pediatric malignancy, accounting for 8%-10% of childhood malignant tumors.[1] NB originates from primitive neural crest cells and can occur anywhere along the sympathetic nerve chain, with nearly half occurring in the adrenal glands.[2] The tumor can metastasize through the lymphatic or hematogenous systems, and approximately 50% of children have local or distant

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metastases at the time of diagnosis.[3] NB exhibits significant heterogeneity, capable of spontaneous regression, transformation into benign ganglioneuroma, or continued progression leading to death, depending on various clinical and biological risk factors.[4]

Currently, clinical treatment regimens generally include surgery, chemotherapy, radiotherapy, immunotherapy, or stem cell transplantation as part of comprehensive treatment.[5] With continuous improvements in diagnostic and therapeutic methods, the overall survival rate (OS) of children has significantly improved. However, the prognosis for children with metastatic NB remains poor.[6] Studies have shown that among children with NB who are older than 18 months and have metastases, only about 50% can be cured despite multiple treatments.[7] Therefore, selecting a reliable and precise tumor staging and prognostic evaluation system, and based on this, choosing appropriate treatment plans is crucial for improving the survival rate and quality of life of these children.

In recent years, the detection of circulating tumor cells (CTCs) in peripheral blood, as an emerging molecular biological "liquid biopsy" technology, has received widespread attention.[8] CTCs are tumor cells shed from primary or secondary tumors into the bloodstream, easy to obtain, and provide comprehensive biological information, including RNA, genomic epigenetic markers, lipids, and proteins.[9] This information can reflect the tumor status and burden level in real time, making it significant for tumor diagnosis, screening, long-term monitoring, and personalized treatment guidance.[10] Compared to traditional tissue biopsies, lymph node biopsies, and bone marrow punctures, CTCs detection is less invasive, simpler to operate, can be collected multiple times, and has high clinical application value.[11] Currently, CTCs detection is mainly used in adult tumors such as prostate cancer,[12] rectal cancer,[13] small cell lung cancer,[14] and breast cancer,[15] but its application in pediatric solid tumors has not yet reached the level of adults. As a common pediatric solid tumor with abundant clinical samples, NB has great research prospects and value.

The innovation of this study lies in introducing the emerging "liquid biopsy" technology of CTCs detection, providing more comprehensive and real-time tumor information through the detection of tumor cells in the blood. This method is simpler and less invasive compared to traditional tissue biopsies, lymph node biopsies, and bone marrow punctures, and can be collected multiple times to achieve dynamic monitoring. By analyzing the relationship between CTCs expression levels and the survival rate of children with NB, this study provides potential observation indicators for clinical treatment and prognosis.

Methods and materials

Sample source

This retrospective analysis included children with NB who received comprehensive treatment at Baoji Third Hospital from September 2020 to October 2022.

Inclusion and exclusion criteria

Inclusion Criteria: Children who met the criteria for stages III and IV according to the INSS staging system;[16] confirmed diagnoses of NB by pathological examination; no prior treatment before this study; complete clinical data.

Exclusion Criteria: Children with an expected survival time of less than 6 months; children with other malignant tumors; children with severe congenital diseases; children lost to follow-up or with incomplete follow-up data.

Sample collection

A total of 224 samples meeting the inclusion criteria were collected, and 80 samples were excluded according to the exclusion criteria, resulting in 144 samples that met the requirements for this study.

Clinical data collection

Clinical data were obtained from the children's electronic medical records and outpatient follow-up records. Baseline data included age, sex, efficacy, maximum tumor diameter, degree of differentiation, tumor stage, and risk level. Efficacy was categorized based on the Expert Consensus on the Diagnosis and Treatment of Childhood Neuroblastoma as remission (including complete remission or partial remission) or progression (including stable disease or worsening disease). The maximum tumor diameter was measured and classified as either <10 cm or ≥10 cm. The degree of tumor differentiation was assessed pathologically using the Shimada classification, which defines three subtypes: undifferentiated, poorly differentiated, and differentiating subtypes. The undifferentiated subtype indicates the highest malignancy, the poorly differentiated subtype shows significant malignancy, and the differentiating subtype suggests lower malignancy. Tumor stage was determined according to the INSS, including stage III and stage IV. According to the expert consensus, the definition of the intermediate-risk group included the following conditions: age <1 year, MYCN unamplified, stage 3; age >1 year, MYCN unamplified and INPC of a favorable prognostic type, stage 3; age <1 ½ years, MYCN unamplified, stage 4; MYCN unamplified and DNA diploid, stage 4; MYCN unamplified and INPC of a favorable prognostic type, stage 4S. The high-risk group was defined to include: age ≥1 year, MYCN amplification and INPC of poor prognostic type, stage 2; age ≥1 year, MYCN amplification, stage 3; age ≥1 year, MYCN nonamplified but INPC of poor prognostic type, stage 3; age ≥1 year, MYCN amplification, stage 4; age <1 year, MYCN amplification, stage 4; all patients with stage 4 who were ≥1 year old, and all patients with MYCN-amplified, stage 4S patients.

CTCs detection

In the morning on an empty stomach, 5 mL of peripheral venous blood was drawn from the children and placed in anticoagulant-containing blood collection tubes to ensure thorough mixing with the anticoagulant to prevent coagulation. These anticoagulant blood samples were stored in a 4 °C environment and tested within 12 h. Red blood cell lysis buffer was added to lyse the red blood cells, and the released matrix was removed by centrifugation (10 cm, 800 rpm, 5 min). The collected white blood cells and tumor cells were subjected to immunomagnetic bead technology to adsorb the

white blood cells, thereby enriching the tumor cells. Finally, CTCs were identified and counted using an automated circulating tumor live cell capture system and immunofluorescence *in situ* hybridization technology.

Treatment plan

All patients followed the standard treatment guidelines of the "Expert Consensus on the Diagnosis and Treatment of Pediatric Neuroblastoma".[17] The specific treatment plan included induction chemotherapy, surgical resection, and postoperative chemotherapy. During the induction chemotherapy phase, patients received 4 cycles of chemotherapy with a regimen consisting of cyclophosphamide (1,000 mg/m^2/day, intravenously, for 2 consecutive days), vincristine (0.05 mg/kg/day, intravenously, once a week), and doxorubicin (30 mg/m^2/day, intravenously, for 2 consecutive days), with each cycle lasting 21 days. After completing induction chemotherapy, if the tumor size was significantly reduced and the condition was stable, patients underwent surgical resection aimed at complete tumor removal. Post-surgery, patients continued with postoperative chemotherapy, receiving 4 cycles of etoposide (100 mg/m^2/day, intravenously, for 3 consecutive days) and cisplatin (60 mg/m^2/day, intravenously, for 2 consecutive days), with each cycle lasting 21 days.

Follow-up

Patients were followed up postoperatively, and the condition of NB patients was assessed according to the efficacy evaluation criteria of the national expert consensus, with complete response and partial response classified as Mitigation. Cases that did not meet the above criteria were classified as Progress. Follow-up continued until June 2024, with follow-ups every 3 months in the first year post-treatment, and every 6 months thereafter.

Determination of optimal cutoff value for CTCs expression

The optimal cutoff value for CTCs expression levels was determined using X-tile software. This software identifies the cutoff point that maximizes the chi-square value in Kaplan-Meier survival analysis, ensuring the best distinction between high and low expression groups based on survival outcomes.

Outcome measurement

1. Analyze the relationship between CTCs expression levels and survival in children with NB using X-tile software to determine the optimal cutoff value. 2. Divide children into high and low expression groups based on the optimal cutoff value from X-tile software and compare the differences in baseline data between the two groups. 3. Analyze the expression levels of CTCs in Efficacy, Maximum tumor diameter, Degree of differentiation, and Tumor stage, and evaluate their diagnostic value.

4. Use Cox regression analysis to identify independent prognostic factors affecting overall survival (OS) in children with NB. 5. Construct a Nomogram model based on independent prognostic factors from Cox regression, and use time-dependent ROC curve analysis to evaluate the model's value in predicting 1-year and 2-year survival.

Statistical analysis

Statistical analysis was performed using SPSS 26.0 software. Measurement data are expressed as mean ± standard deviation (mean ± sd), and comparisons between groups were made using an independent samples t-test. Count data are expressed as percentages or rates (%), and the chi-square test was used for comparisons. The ROC curve was used to analyze the diagnostic value of CTCs in Efficacy, Maximum tumor diameter, Degree of differentiation, and Tumor stage. Cox regression analysis was used to identify independent prognostic factors affecting overall survival (OS) in children with NB. The Nomogram was constructed using the rms package in R software (version 4.3.2). ROC curve analysis was performed using the pROC package, and time-dependent ROC curve analysis was conducted using the timeROC package. The optimal cut-point for predicting outcomes was determined using the Youden Index, and this has been noted in the statistical section. Kaplan-Meier (K-M) curve analysis was carried out using the survival and survminer packages. All visualizations were done using ggplot2. A p-value of <0.05 was considered statistically significant.

Results

The relationship between CTCs expression levels and survival in children with NB

By the follow-up deadline in June 2024, 43 children were still alive, with a survival rate of 29.86%. The average survival time of the children was 22 months (95% CI: 20.23 ~ 23.20). The prognostic value of CTCs in NB was analyzed using X-tile software. The results showed that when the optimal cutoff value was <14 (CTCs/mL), the prognosis of the children significantly improved (Figure 1A-B).

Comparison of baseline data based on CTCs expression levels

According to the optimal cutoff values obtained from X-tile software, the children were divided into high-and low-expression groups. Further comparison of the baseline data between the two groups revealed that the number of children who developed progressive disease ($p < 0.001$), Maximum tumor diameter ≥10cm ($p = 0.004$), undifferentiated subtype ($p = 0.034$), and stage IV ($p = 0.007$) in the high expression group was significantly higher than in the low expression group, indicating statistical significance (Table 1). There was no statistical significance in age, sex, and risk level between the groups ($p > 0.05$).

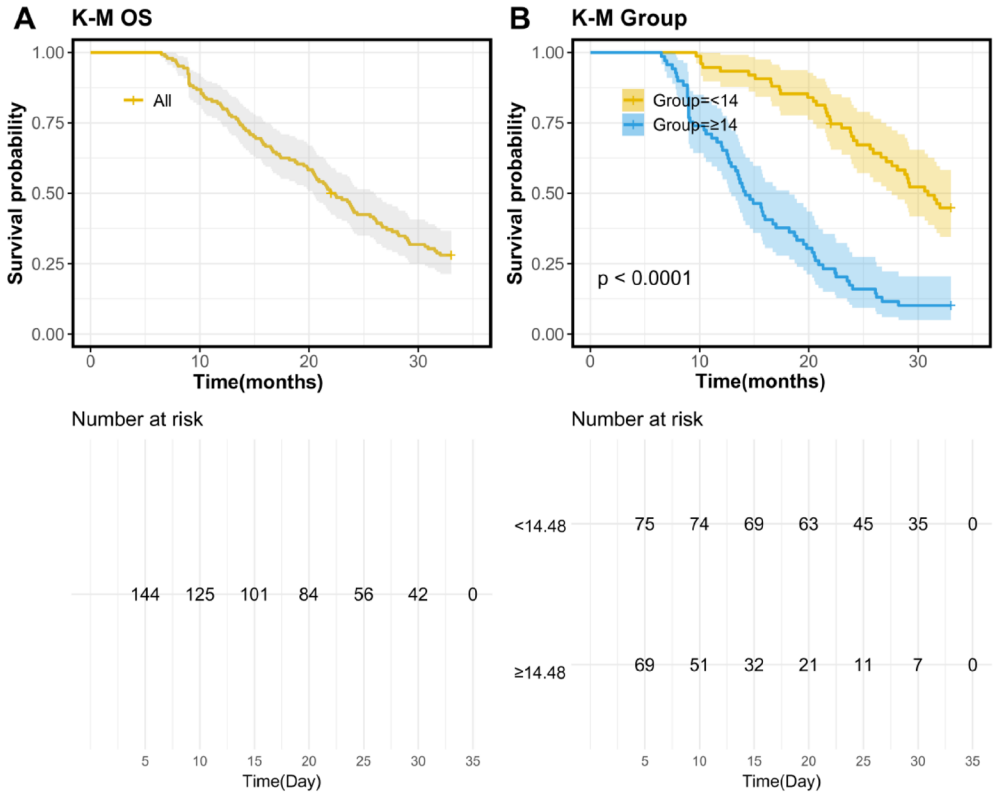

Figure 1. CTCs Expression levels and K-M survival curves in children with NB. A. K-M survival curve of overall survival in children with NB. B. K-M survival curve of CTCs expression levels in children with NB.

Note: Neuroblastoma (NB) and circulating tumor cells (CTCs).

CTCs expression in efficacy, maximum tumor diameter, degree of differentiation, and tumor stage

Baseline data analysis revealed that CTCs were associated with Efficacy, Maximum tumor diameter, Degree of differentiation, and Tumor stage. Therefore, further comparison of CTCs expression levels in different baseline data was conducted. The results showed that CTCs in children with Progress were higher than those in children with Mitigation, with statistical significance ($p < 0.001$, Figure 2A); children with Maximum tumor diameter <10cm had lower CTCs than those with ≥10cm, with statistical significance ($p < 0.001$, Figure 2B); there was no difference in CTCs among children with different degrees of differentiation ($p = 0.148$, Figure 2C); children with stage IV had higher CTCs than those with stage III, with statistical significance ($p = 0.036$, Figure 2D).

Diagnostic value of CTCs in efficacy, maximum tumor diameter, degree of differentiation, and tumor stage

The above study determined that CTCs expression was related to Efficacy, Maximum tumor diameter, Degree of differentiation, and Tumor stage. Therefore, separate ROC

Table 1. Relationship between CTCs expression levels and baseline data.

Factors		CTCs expression levels		χ^2 Value	*p* value
		High expression (*n* = 69)	Low expression (*n* = 75)		
Age					
	≥18 months	31	43	2.214	0.137
	<18 months	38	32		
Sex					
	Male	43	53	1.127	0.288
	Female	26	22		
Efficacy					
	Progress	40	17	18.73	<0.001
	Mitigation	29	58		
Maximum tumor diameter					
	≥10cm	51	38	8.227	0.004
	<10cm	18	37		
Degree of differentiation					
	undifferentiated subtype	29	19	4.508	0.034
	Poorly differentiated and differentiating subtypes	40	56		
Tumor stage					
	Stage IV	43	30	7.162	0.007
	Stage III	26	45		
Risk level					
	High risk	52	53	0.401	0.526
	Medium risk	17	22		

Note: Circulating tumor cells (CTCs).

curves for CTCs in Efficacy, Maximum tumor diameter, Degree of differentiation, and Tumor stage were plotted. The results showed that the area under the curve (AUC) for Efficacy, Maximum tumor diameter, Degree of differentiation, and Tumor stage were 0.703, 0.669, 0.574, and 0.598, respectively (Figure 3, Table 2). Among them, CTCs had the highest AUC in distinguishing clinical efficacy, while the AUC for distinguishing tumor stage and degree of differentiation was the lowest.

Prognostic factors affecting OS in children with NB

In this study, Cox regression analysis was used to screen prognostic factors affecting OS in children with NB. Univariate analysis showed that CTCs ($p < 0.001$, OR = 4.221), Efficacy ($p < 0.001$, OR = 2.395), Maximum tumor diameter ($p < 0.001$, OR = 3.43), and Degree of differentiation ($p = 0.01$, OR = 1.69) were prognostic factors affecting the prognosis of children (Table 3). Multivariate Cox regression analysis showed that CTCs ($p < 0.001$, OR = 2.958), Efficacy ($p = 0.005$, OR = 1.814), and Maximum tumor diameter ($p < 0.001$, OR = 2.586) were independent prognostic factors affecting OS in children with NB (Table 4, Figure 4).

Construction of a nomogram model based on independent prognostic factors

At the end of the study, a visual Nomogram model was constructed based on independent prognostic factors. The model included Efficacy, Maximum tumor diameter,

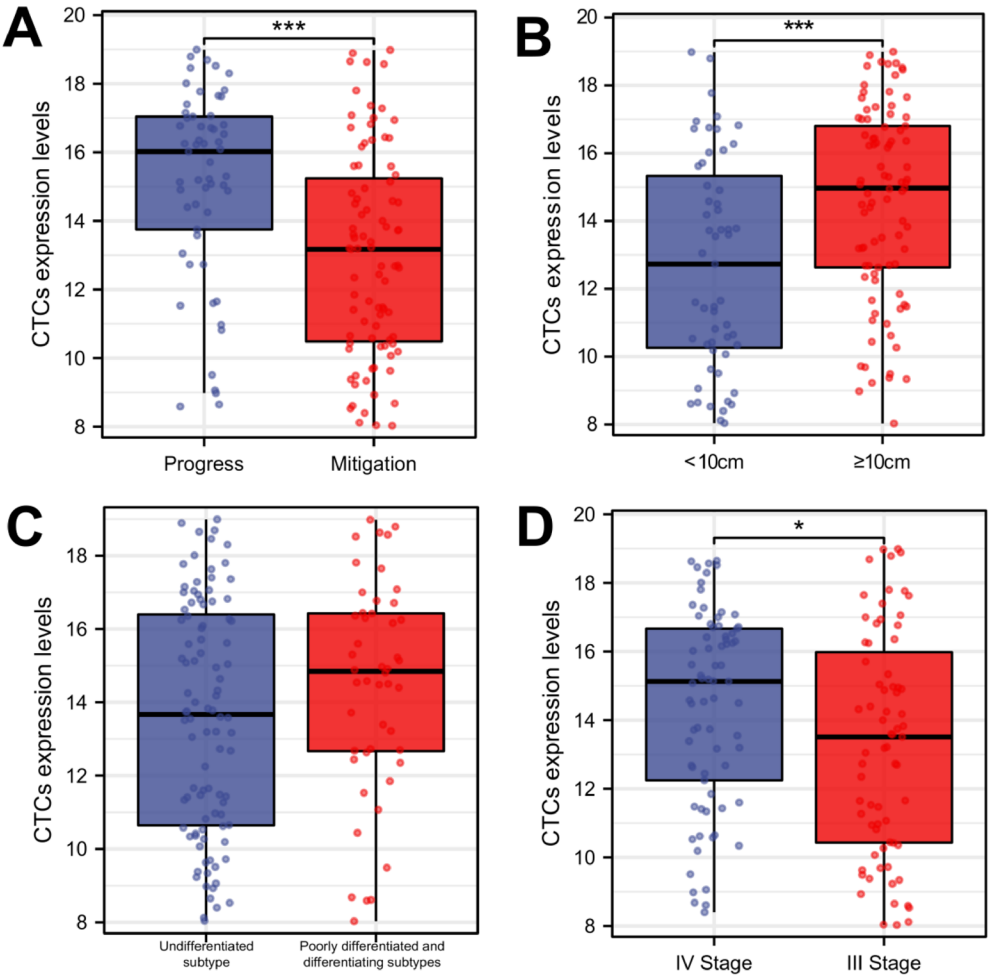

Figure 2. CTCs Expression in efficacy, maximum tumor diameter, degree of differentiation, and tumor stage. A. Comparison of CTCs expression in efficacy. B. Comparison of CTCs expression in maximum tumor diameter. C. Comparison of CTCs Expression in degree of differentiation. D. Comparison of CTCs expression in tumor stage
Note: Circulating tumor cells (CTCs).

and CTCs. Among them, CTCs were strongly correlated with patients' OS, while Efficacy and Maximum tumor diameter were related to tumor OS (Figure 5A). Furthermore, time-dependent ROC curve analysis showed that the model's AUC for predicting 1-year and 2-year survival was 0.747 and 0.802, respectively, indicating that the model has a certain clinical value in predicting patients' survival (Figure 5B).

Discussion

Understanding the molecular mechanisms of NB tumor progression helps evaluate tumor growth, invasion, and metastasis, guiding early treatment and improving the therapeutic outcomes of malignant tumors.[18] CTCs are tumor cells that enter the circulatory system after shedding from primary or secondary tumors. Under appropriate

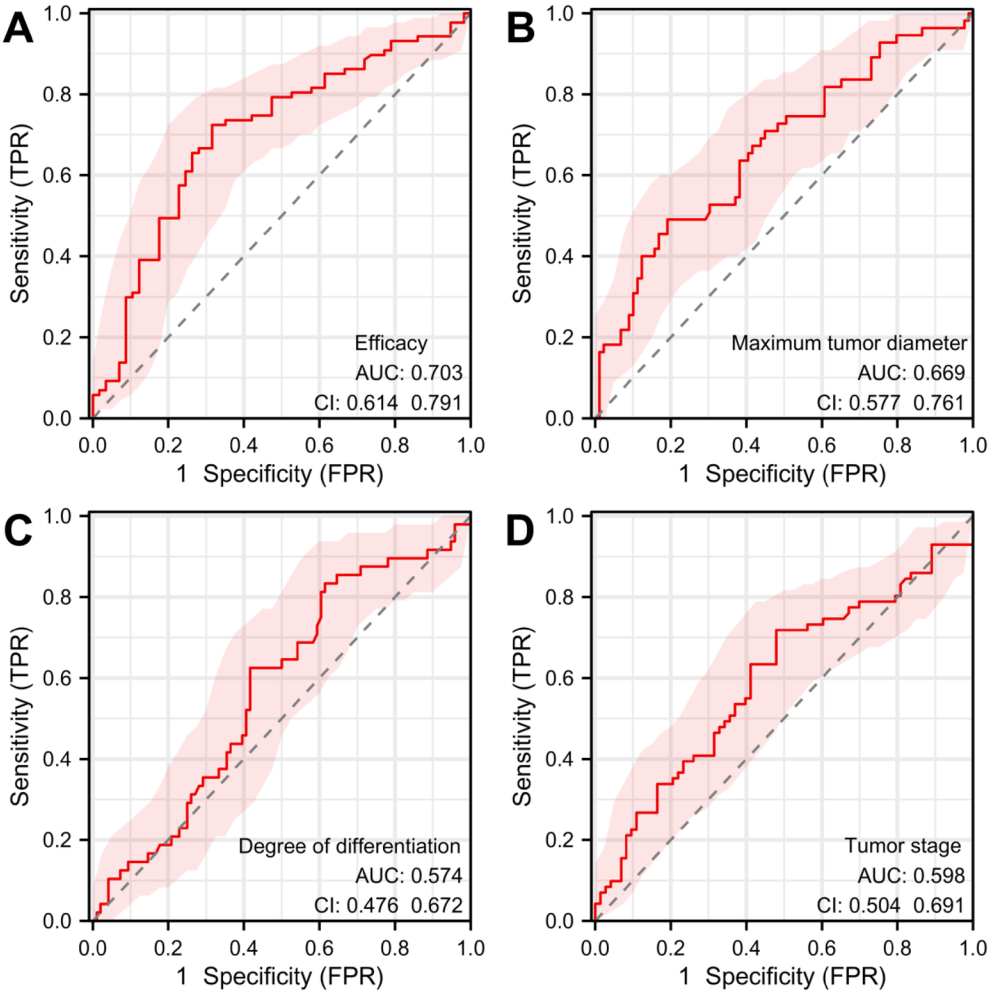

Figure 3. Diagnostic value of CTCs in efficacy, Maximum tumor diameter, degree of differentiation, and tumor stage. A. ROC curve of CTCs in efficacy. B. ROC curve of CTCs in maximum tumor diameter. C. ROC curve of CTCs in the degree of differentiation. D. ROC curve of CTCs in tumor stage
Note: Circulating tumor cells (CTCs) and receiver operating characteristic (ROC).

Table 2. ROC parameters.

Metric	Efficacy	Maximum tumor diameter	Degree of differentiation	Tumor stage
AUC	0.703	0.669	0.574	0.598
95%CI	0.614 – 0.703	0.577 – 0.669	0.476 – 0.574	0.504 – 0.598
specificity	0.724	0.491	0.385	0.718
sensitivity	0.684	0.809	0.833	0.521
Youden index	0.408	0.3	0.219	0.239
Cut off	14.845	11.655	11.755	15.06
Accuracy	0.708	0.688	0.535	0.618
Precision	0.619	0.72	0.404	0.655
F1 Score	0.65	0.762	0.544	0.58

Note: Circulating tumor cells (CTCs) and area under curve (AUC).

Table 3. Univariate cox regression.

Factor	Beta	StdErr	*p* value	HR	95% CI Lower Limit	95% CI Upper Limit
CTCs	1.440	0.213	<0.001	4.221	2.782	6.404
Age	0.060	0.199	0.763	1.062	0.718	1.570
Sex	−0.041	0.211	0.847	0.960	0.635	1.451
Efficacy	0.873	0.200	<0.001	2.395	1.617	3.548
Maximum tumor diameter	1.232	0.237	<0.001	3.430	2.154	5.461
Degree of differentiation	0.525	0.205	0.010	1.690	1.131	2.525
Tumor stage	0.296	0.200	0.138	1.345	0.909	1.990
Risk level	−0.008	0.222	0.973	0.992	0.642	1.534

Note: Circulating tumor cells (CTCs).

Table 4. Multivariate Cox regression.

Factor	Beta	StdErr	*p* value	HR	95% CI Lower limit	95% CI Upper limit
CTCs	1.084	0.230	<0.001	2.958	1.884	4.643
Efficacy	0.595	0.211	0.005	1.814	1.199	2.744
Maximum tumor diameter	0.950	0.250	<0.001	2.586	1.584	4.222
Degree of differentiation	0.166	0.210	0.428	1.181	0.783	1.781

Note: Circulating tumor cells (CTCs).

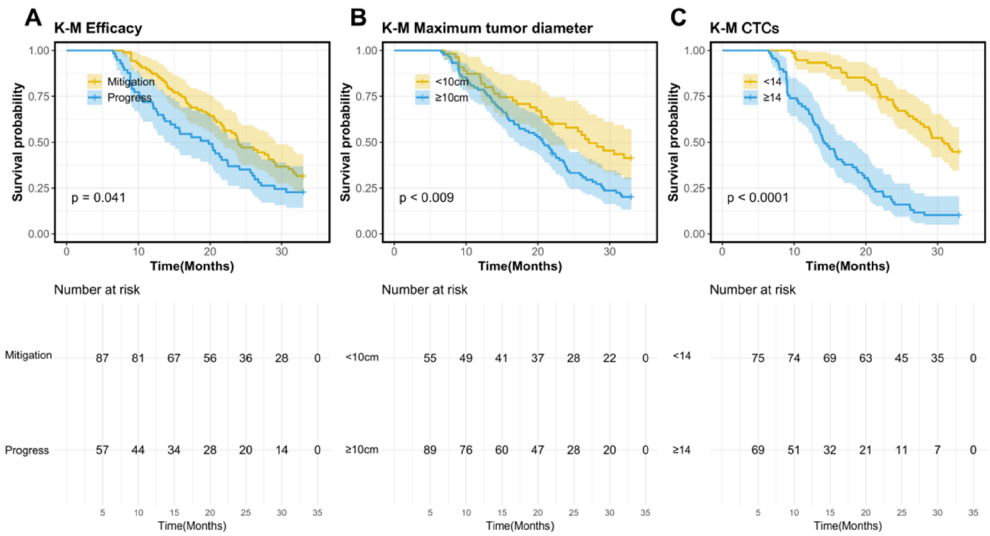

Figure 4. K-M survival curves of independent prognostic factors. A. K-M survival curve of different efficacy in children. B. K-M survival curve of different maximum tumor diameters in children. C. K-M survival curve of different CTCs expression levels in children.
Note: Circulating tumor cells (CTCs).

microenvironmental conditions, a small portion of CTCs can evolve into metastatic tumors.[19] Therefore, CTCs detection can be used for early tumor diagnosis. Additionally, measuring the concentration of CTCs in a patient's blood can reflect the tumor burden, aiding in tumor diagnosis, chemotherapy efficacy, and prognostic evaluation.[20] In this

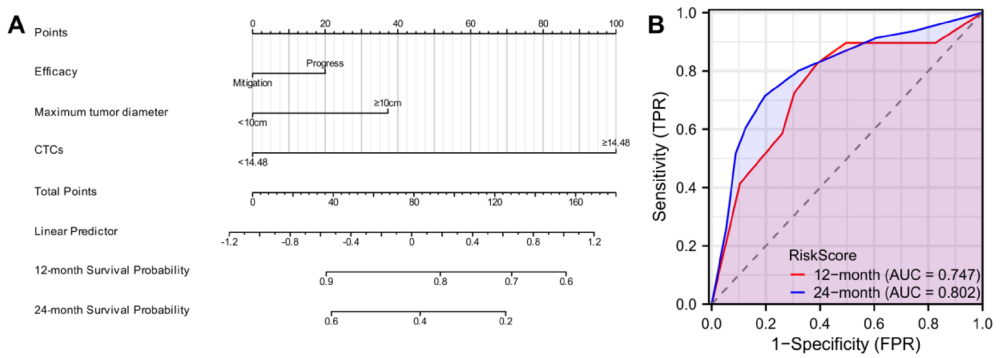

Figure 5. Construction of a nomogram model based on independent prognostic factors. A. Nomogram model of independent prognostic factors. B. Time-dependent ROC curve of the Nomogram Model in Predicting 1-year and 2-year Survival.
Note: Receiver operating characteristic (ROC).

study, we examined the relationship between CTCs levels in children with NB and their prognosis. The results showed that when CTCs were <14, the prognosis of the children significantly improved. Further analysis revealed that children with high CTCs expression had significantly higher numbers of Progress cases, Maximum tumor diameter ≥10cm, undifferentiated subtype cases, and stage IV cases compared to those with low CTCs expression. These results suggest that high CTCs levels may indicate a higher rate of tumor cell shedding and greater metastatic potential. Shed tumor cells entering the circulatory system may form new metastatic sites in other parts of the body, leading to disease progression and deterioration.[21] Therefore, CTCs detection has important clinical application value, allowing physicians to assess tumor aggressiveness and metastatic risk and develop more targeted treatment plans.

Previous studies by Zhu et al.[22] have shown that CTC-leukocyte aggregates appearing before and after two cycles of chemotherapy and the total count of CTCs at the end of chemotherapy are strong predictors of prognosis in patients with small cell lung cancer receiving first-line treatment. Additionally, Yan et al.[23] reported that positive CTCs are an independent predictor of poor prognosis in patients with gallbladder adenocarcinoma after resection. Preoperative CTC detection may play an important role in guiding treatment strategies for these patients. Furthermore, Li et al.[24] found that CTCs have a guiding effect on adjuvant therapy for patients with early-stage endometrial cancer post-surgery. These findings are consistent with the conclusions of this study, suggesting that CTCs detection has significant value in prognostic evaluation and treatment strategy formulation across various tumor types.

To further determine the relationship between CTCs and baseline data, we compared the expression of CTCs in different baseline data categories, such as Efficacy, Maximum tumor diameter, Degree of differentiation, and Tumor stage. The results showed that CTCs levels were higher in children with Progress than those with Mitigation, higher in children with Maximum tumor diameter ≥10cm than those with <10cm, and higher in stage IV children than stage III children, but there were no difference in CTCs levels among children with different degrees of differentiation. The consistency of Efficacy, Maximum tumor diameter, and Tumor stage with previous results was observed, while the lack of difference in CTCs levels among different degrees of

differentiation might be due to the small sample size and the varying frequency distribution of CTCs levels among patients with different degrees of differentiation. We hope to collect more samples in future studies to improve these results. Interestingly, further ROC curve analysis revealed that pretreatment CTCs have high clinical value in assessing efficacy, with an AUC of 0.703. We speculate that this is because high CTCs levels may indicate a higher tumor burden and invasiveness, which in turn affects treatment outcomes.[25] Additionally, high CTCs levels may reflect a higher rate of tumor cell shedding, associated with tumor biological behavior.[26] Furthermore, high-shedding tumors are less sensitive to treatment, affecting efficacy evaluation. For instance, the study by Gradilone et al.[27] found that patients with positive CTCs and those with CTCs expressing two or more multidrug resistance-related proteins had shorter progression-free survival (PFS).

Finally, we used Cox regression analysis to identify independent prognostic factors affecting OS in children with NB. The results showed that CTCs, Efficacy, and Maximum tumor diameter were independent prognostic factors affecting OS in children with NB. Efficacy reflects the patient's direct response to treatment, indicating whether the treatment successfully controls the tumor. Effective treatment can significantly reduce tumor burden, delay disease progression, and prolong survival.[28] Efficacy also indicates whether the disease is stable, mitigated, or progressing, which is crucial for long-term survival. Maximum tumor diameter is a direct indicator of tumor burden; larger tumors usually indicate a higher tumor burden and more complex treatment challenges.[29] Larger tumors are more likely to metastasize and invade, leading to poor prognosis. Larger tumors generally represent a later stage and higher severity of the disease, indicating greater treatment challenges and lower chances of cure.[30] CTCs reflect the presence and quantity of tumor cells shed from primary or secondary sites into the circulatory system, directly indicating metastatic potential.[31] High CTCs levels suggest that tumor cells are prone to shedding and metastasis, indicating higher metastatic risk and poorer prognosis.[32] Additionally, Kreissman et al.[33] conducted a study exploring whether immunomagnetic purging of peripheral blood stem cells (PBSC) in high-risk neuroblastoma patients could improve event-free survival (EFS) and overall survival (OS). Although this purging process reduced the tumor burden in the transplanted cells, the results showed that purging did not significantly improve survival rates, as there was no significant difference in the 5-year EFS and OS between the purged and non-purged groups. This finding suggests that while reducing tumor cell burden is an important therapeutic goal, stem cell purging alone may not significantly improve the prognosis of high-risk patients. Similarly, our study demonstrates that CTCs detection, as a noninvasive biomarker evaluation tool, has the potential to identify high-risk neuroblastoma patients. CTCs detection provides a real-time method for monitoring tumor burden, which may help in the early identification of disease progression or remission, thereby optimizing treatment strategies and potentially improving prognosis. Therefore, CTCs detection could become an important tool in the management of neuroblastoma, especially in high-risk patients. However, as shown by Kreissman et al., a single technique or strategy may not be sufficient to significantly improve prognosis, and combining CTCs detection with other therapeutic approaches may be an important direction for future research. These indicators not only reflect tumor burden and biological behavior but also provide important information on treatment

response and prognosis, helping clinicians develop more precise treatment strategies to improve long-term survival rates in children.

Based on these independent prognostic factors, we constructed a visual Nomogram model and found that the model's AUC for predicting 1-year and 2-year survival was 0.747 and 0.802, respectively. This suggests that CTCs, Efficacy, and Maximum tumor diameter are not only important prognostic indicators but can also provide reliable survival prediction tools through the Nomogram model, offering strong support for clinicians in developing individualized treatment plans and prognostic evaluations.

However, this study has certain limitations. First, the sample size was relatively small, including only 144 children, which may affect the stability and generalizability of the statistical results. Second, the study was conducted at a single center, potentially introducing regional and selection biases. Additionally, as a retrospective study, there is a risk of selection and information biases, potentially affecting the accuracy of the results. Furthermore, while we used tumor diameter as the sole determinant for tumor burden in this study, we did not account for metastatic tumor burden, such as by using Curie scores based on MIBG scans prior to transplant. The absence of this data represents a deficiency, as it could have provided additional insight into the correlation between metastatic tumor burden and CTC. Lastly, the follow-up period was relatively short, possibly insufficient for a comprehensive evaluation of long-term prognosis. We hope that future studies can address these limitations by expanding the sample size, collaborating with multiple centers, designing prospective studies, incorporating metastatic tumor burden assessments, and extending the follow-up period to provide more comprehensive and reliable prognostic evaluations, ultimately improving clinical treatment outcomes.

Conclusion

This study demonstrates that CTCs, Efficacy, and Maximum tumor diameter are independent prognostic factors affecting overall survival (OS) in children with advanced NB. These findings suggest that CTCs, Efficacy, and Maximum tumor diameter are not only important prognostic indicators but can also provide reliable survival prediction tools through the Nomogram model, offering strong support for clinicians in developing individualized treatment plans and prognostic evaluations.

Ethical approval and consent to participate

This study has been approved by the Ethics Committee of Baoji Third Hospital. Due to the retrospective observational nature of this study, a clinical trial registration exemption policy has been implemented.

Authors' contributions

Junhua Tuo, Zhi Zhao, and Xuan He have significantly contributed to the conception and design of the manuscript, while Xiaoning Ma and Zhengsheng Liu have made valuable contributions in the acquisition, analysis, and interpretation of the data. The entire authorship team actively participated in drafting the manuscript, with Baogang Yang and Meng Zhang providing critical revisions. Finally, all authors carefully reviewed and approved the final version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data for this study has been uploaded to "Science Data Bank" and can be obtained through the link: https://www.scidb.cn/anonymous/SVpGRjMy. If more detailed data is needed, please contact the corresponding author, and we will provide the corresponding data according to policy permission.

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