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# Polo-like Kinase 4: the Variation During Therapy and its Relation to Treatment Response and Prognostic Risk Stratification in Childhood Acute Lymphoblastic Leukemia Patients

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**Summary:** Polo-like kinase 4 (PLK4) plays an essential role in the tumorigenesis of some blood malignancies; consequently, we hypothesized that PLK4 might serve as a potential biomarker in childhood acute lymphoblastic leukemia (ALL) patients. Therefore, this study investigated the expression of PLK4 and its clinical relevance in childhood ALL patients. Bone marrow specimens were collected from 95 childhood ALL patients and 20 primary immune thrombocytopenia patients (as controls), and their PLK4 expression (reverse transcription-quantitative polymerase chain reaction) was measured after enrollment. Besides, the PLK4 expression in childhood ALL patients was also determined at day 15 after the initiation of induction therapy (D15). PLK4 was increased in childhood ALL patients compared with controls (2.830 (interquartile range (IQR): 1.890-3.660) versus 0.976 (IQR: 0.670-1.288), $P \leq 0.001$). PLK4 at diagnosis was elevated in T cell acute lymphoblastic leukemia patients than in B cell acute lymphoblastic leukemia patients ($P = 0.027$). Besides, PLK4 at diagnosis was positively linked with the Chinese Medical Association risk stratification ($P = 0.016$), but not with prednisone response ($P = 0.077$) or bone marrow response ($P = 0.083$). In addition, PLK4 was decreased at D15 after treatment compared with at diagnosis ($P \leq 0.001$). Interestingly, PLK4 at D15 ($P = 0.033$) was elevated in T cell acute lymphoblastic leukemia patients than in B cell acute lymphoblastic leukemia patients. Furthermore, increased PLK4 at D15 was associated with poor prednisone response ($P = 0.018$), poor bone marrow response ($P = 0.034$), and increased the Chinese Medical Association risk stratification ($P = 0.015$). In terms of prognosis, high PLK4 was associated with shorter event-free survival ($P = 0.020$), whereas it was not related to the overall survival ($P = 0.135$). In conclusion, PLK4 has the potential as a biomarker for treatment response and prognostic risk stratification of childhood ALL patients.

**Key Words:** polo-like kinase 4, childhood acute lymphoblastic leukemia, treatment response, CMA risk stratification, survival

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Childhood acute lymphoblastic leukemia (ALL) is the most prevalent cancer in children and is caused by the malignant transformation and hyperproliferation of lymphoid progenitor cells (B cells and T cells).[1–4] In recent years, with the development of diverse specific risk-stratification guidelines and the spread of standardized treatment, childhood ALL patients can achieve a favorable prognosis (5-year survival rate exceeding 90%).[5–9] However, some childhood ALL patients lack treatment response and are at high risk of relapse.[10] Therefore, finding additional biomarkers could help monitor treatment response and prognostic risk stratification in childhood ALL patients. Clinicians can re-modulate the intensity of treatment to improve the prognosis of these childhood ALL patients.

Polo-like kinase 4 (PLK4), located on chromosome 4q27-28, is a unique member of the PLK family that has been reported to play an essential role in the initiation and progression of several blood malignancies (including diffuse large B-cell lymphoma, ALL, multiple myeloma, etc.)[11–16] because of its particular structure (triple polo box architecture). For instance, a study supported that PLK4 enhanced cell cycle aberrations and further led to uncontrolled cell proliferation and DNA damage in diffuse large B-cell lymphoma.[15] As for ALL, a previous study found that PLK4 causes mitotic infidelity (including polyploidy and cytokinesis failure) via the interaction with Janus kinase 2 (JAK2), suggesting that PLK4 may be involved in the pathogenesis of ALL.[12] Consequently, we hypothesized that PLK4 might have potential as a biomarker in childhood ALL patients. However, the detailed implication of PLK4 in childhood ALL patients remains unanswered.

Therefore, in this study, PLK4 was detected in the bone marrow specimens from 95 childhood ALL patients and another 20 primary immune thrombocytopenia patients (as controls) to investigate the aberrant expression of PLK4 and its clinical relevance in childhood ALL patients.

## MATERIALS AND METHODS

### Subjects

Ninety-five newly diagnosed childhood ALL patients were consecutively enrolled in this study from January 2017 to December 2020. The inclusion criteria were as follows: (i) newly diagnosed as ALL; (ii) aged less than 16 years; (iii) about to receive treatment under the Chinese Children's Leukemia Group-ALL 2008 treatment strategy;[17] (iv) willing to provide bone marrow specimen for study use; (v) willing to be followed up regularly; and (vi) willing to provide the written informed consent. The exclusion criteria

were set as: (i) diagnosis of recurrent or refractory ALL; (ii) had a prior history of other hematologic malignancies or solid tumors; (iii) previous chemotherapy, radiation, targeted therapy, or immunotherapy; and (iv) moderate to severe dysfunction of the heart, lung, liver, or kidney. In addition, a total of 20 primary immune thrombocytopenia patients who underwent bone marrow aspiration were recruited as controls in the study between 2017 and December 2020, and all controls had matched age and sex to childhood ALL patients. The study was approved by the Ethics Committee of Handan Central Hospital. For patients aged $\leq$12 years, the informed consent was collected from the guardians; for patients aged $\geq$12 years, the informed consent was collected from both the patients and the guardians.

## Data Documents

After admission, clinical characteristics of childhood ALL patients were recorded, including age, sex, height, weight, immunophenotype, French-American-British (FAB) classification, and white blood cell (WBC) count.

## Specimen Collection and Assessment

Bone marrow specimens were collected from controls after admission and from childhood ALL patients at diagnosis and on day 15 after starting induction therapy (D15). The specimens were then immediately placed into liquid nitrogen to further detection of PLK4 expression by reverse transcription-quantitative polymerase chain reaction. Total RNA was extracted with the RNeasy Protect Mini Kit (Qiagen, Germany), and then reserve transcription was completed with the QuantiTect Rev. Transcription Kit (Qiagen, Germany). Afterward, qPCR was implemented by the QuantiNova SYBR Green PCR Kit (Qiagen, Germany). The expression of PLK4 was calculated by the $2^{-\Delta\Delta Ct}$ method, using glyceraldehyde-3-phosphate dehydrogenase as an internal reference. Besides, qPCR primers were designed referring to a previous study.[18]

## Treatment and Assessment

For all childhood ALL patients, prednisone was administered for 7 days before chemotherapy. Then prednisone response was assessed on day 8 and classified as prednisone poor response (PPR) and prednisone good response . PPR was defined as the absolute blast count in peripheral blood $\geq 11 \times 10^9$/L; prednisone good response was defined as the absolute blast count in peripheral blood $\leq 1 \times 10^9$/L blasts.[19] After administration of prednisone, the patients received chemotherapy according to the Chinese Children's Leukemia Group-ALL 2008 treatment strategy, including induction therapy, early intensification, consolidation, delayed intensification, and maintenance.[17] At D15, bone marrow response was assessed, and categorized as M1 ($\leq$5% blasts), M2 ($\geq$15% to $\leq$25% blasts), and M3 ($\geq$125% blasts).[20] In addition, risk stratification was assessed according to the Chinese Medical Association (CMA) recommendations for managing acute lymphoblastic leukemia in children (fourth revision), classified as low risk, intermediate risk, and high risk. Moreover, the patients were followed up every 1 to 2 months in the first year after the last dosage of chemotherapy, every 3 to 6 months in the second year, and every 6 to 12 months thereafter. The final date of follow-up was July 31, 2021. Event-free survival (EFS) and overall survival (OS) were calculated based on the follow-up information. EFS was the duration from the start of chemotherapy to the occurrence of disease progression, disease relapse, or patient's death for any reason. OS was the duration from the start of chemotherapy to the occurrence of a patient's death for any reason.

## CMA Risk Stratification

The risk factors associated with the prognosis of childhood ALL were as follows: (i) aged $\leq$1 year or $>$10 years; (ii) white blood cell (WBC) $\geq 150 \times 10^9$ /L at diagnosis; (iii) diagnosed as central nervous system leukemia or testicular leukemia; (iv) immunophenotype with T cell acute lymphoblastic leukemia; (v) adverse cytogenetic characteristics, such as t(9;22) (BCR-ABL) and t(4;11) (MLL-AF4); (vi) PPR with prednisone response; (vii) M3 of bone marrow response at day 15; (viii) M2 or M3 of bone marrow response at day 33; (ix) minimal residual disease (MRD) $>1 \times 10^{-4}$ at day 33. Patients were stratified into three risk groups: (1) low risk: no risk factors related to the prognosis of childhood ALL; (2) intermediate risk: had any one or more of the following conditions: (i) aged $\leq$1 year or $>$10 years; (ii) WBC $\geq 150 \times 10^9$ /L; (iii) confirmed as central nervous system leukemia or testicular leukemia; (iv) had immunophenotype of T cell ALL; (v) t(1;9) (q23; p13)/*E2A-PBX1* positive; (vi) confirmed as low risk at diagnosis and M3 of bone marrow response at day 15; and (vii) MRD within $1 \times 10^{-4}$- $1 \times 10^{-2}$; at day 33; (3) high risk: had any one or more of the following conditions: (i) t(9;22) (q34; q11.2)/*BCR-ABL1* positive; (ii) t(4;11) (q21; q23) *MLL-AF4* or other *MLL* genes positive; (iii) PPR of prednisone response; (iv) confirmed as low risk at diagnosis and M3 of bone marrow response at day 15; (v) M2 or M3 of bone marrow response at day 33; and (vi) MRD $>1 \times 10^{-2}$ at day 33.[21,22]

## Statistics

Data analysis and graph plotting were completed with the SPSS V.22.0 (IBM Corp.) and GraphPad Prism V.7.01 (GraphPad Software Inc.), respectively. Correspondingly, the Wilcoxon rank-sum test, Student t test, and $\chi^2$ test were used to compare PLK4 expression, age, and sex between childhood ALL patients and controls. A receiver-operating characteristic curve was carried out to assess the performance of PLK4 expression in differentiating different subjects. In childhood ALL patients, the correlation between PLK4 expression and clinical characteristics at diagnosis was assessed using the Wilcoxon rank-sum test or the Spearman rank correlation test. The change of PLK4 expression over time was analyzed using the Wilcoxon signed-rank test. The median expression of PLK4 at diagnosis was 2.830, which divided the patients into PLK4 high expression and PLK4 low expression groups. The difference in EFS and OS between the two groups was elucidated by Kaplan–Meier curves by the log-rank test. Factors affecting EFS and OS were determined by multivariate Cox's proportional hazards regression analysis using the enter method and backward-stepwise method. A *P*-value less than 0.05 indicated statistical significance.

# RESULTS

## Clinical Characteristics of Childhood ALL Patients

Among all 95 childhood ALL patients, 32 (33.7%) were females, and 63 (66.3%) were males with a mean age of $7.3 \pm 2.8$ years (Table 1). In terms of the immunophenotype, 13 (13.7%) patients were diagnosed as T cell ALL (T-ALL), while 82 (86.3%) patients were diagnosed as B cell ALL

**TABLE 1.** Clinical Characteristics

| Items | Childhood ALL Patients (N = 95) |
|---|---|
| Age (years), mean ± SD | 7.3 ± 2.8 |
| Sex, No. (%) | |
| Female | 32 (33.7) |
| Male | 63 (66.3) |
| Immunophenotype, No. (%) | |
| T-ALL | 13 (13.7) |
| B-ALL | 82 (86.3) |
| FAB classification, No. (%) | |
| L1 | 70 (73.7) |
| L2 | 21 (22.1) |
| L3 | 4 (4.2) |
| WBC count, No. (%) | |
| ≤ 50 X10$^9$/L | 76 (80.0) |
| > 50 X10$^9$/L | 19 (20.0) |
| Prednisone response (D8), No. (%) | |
| PGR | 80 (84.2) |
| PPR | 15 (15.8) |
| Bone marrow response (D15), No. (%) | |
| M1 | 75 (78.9) |
| M2 | 16 (16.8) |
| M3 | 4 (4.2) |
| Risk stratification, No. (%) | |
| Low risk | 29 (30.5) |
| Intermediate risk | 47 (49.5) |
| High risk | 19 (20.0) |

ALL indicates acute lymphoblastic leukemia; B-ALL, B cell acute lymphoblastic leukemia; FAB, French-American-British; M1, ≤ 5% blasts; M2, ≥ 15% to ≤ 25% blasts; M3, ≥ 125% blasts; PPR, plasmatic poor respond; SD, standard deviation; T-ALL, T cell acute lymphoblastic leukemia; WBC, white blood cell; PGR, plasmatic good respond.

(B-ALL). Regarding the FAB classification, 70 (73.7%), 21 (22.1%), and 4 (4.2%) patients were divided into L1, L2, and L3, respectively. In addition, 76 (80.0%) patients had a WBC count ≤50 ×10$^9$/L, whereas another 19 (20.0%) patients had a WBC count >50 ×10$^9$/L. Furthermore, 29 (30.5%), 47 (49.5%), and 19 (20.0%) patients were categorized as low risk, intermediate risk, and high risk according to CMA risk stratification, correspondingly. The detailed clinical characteristics of childhood ALL patients are listed in Table 1.

In addition, the controls included 7 (35.0%) females and 13 (65.0%) males with a mean age of 7.3 ± 2.9 years (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/JPHO/A546). Notably, age ($P = 0.982$) and sex ($P = 0.910$) did not differ between childhood ALL patients and controls.

## PLK4 in Childhood ALL Patients and Controls

PLK4 was increased in childhood ALL patients compared with controls (2.830 (interquartile range (IQR): 1.890-3.660) versus 0.976 (IQR: 0.670-1.288), $P \leq 0.001$) (Fig. 1A). In addition, PLK4 exhibited a good value in differentiating childhood ALL patients from controls (area under the curve): 0.909, 95% confidence interval (CI): 0.847-0.971) (Fig. 1B).

## Correlation of PLK4 with Clinical Characteristics of Childhood ALL Patients

PLK4 at diagnosis was not associated with prednisone response ($P = 0.077$) or bone marrow response ($P = 0.083$), but elevated PLK4 at diagnosis was correlated with increased CMA risk stratification ($P = 0.016$) in childhood

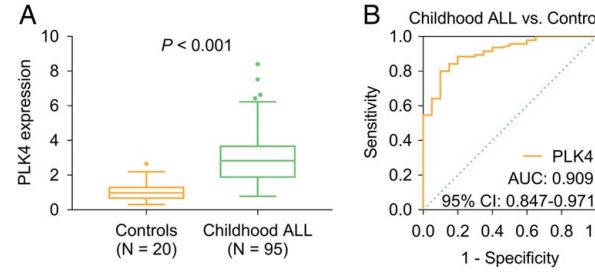


**FIGURE 1.** PLK4 was upregulated in childhood ALL patients than controls. Comparison of PLK4 expression in childhood ALL patients and controls (**A**). The value of PLK4 in distinguishing childhood ALL patients from controls (**B**).ALL indicates acute lymphoblastic leukemia; PLK4, polo-like kinase 4. full color online

ALL patients (Figs. 2A–C). In addition, PLK4 at D15 after treatment was decreased compared with that at diagnosis ($P \leq 0.001$) (Fig. 2D). Differently, increased PLK4 at D15 after treatment was associated with PPR ($P = 0.018$), poor bone marrow response ($P = 0.034$), and increased CMA risk stratification ($P = 0.015$) in childhood ALL patients (Figs. 2EG).

Furthermore, elevated PLK4 at diagnosis ($P = 0.027$) and D15 after treatment ($P = 0.033$) were linked with T-ALL immunophenotype in childhood ALL patients (Table 2). However, PLK4 at diagnosis ($P = 0.198$) and D15 after treatment ($P = 0.246$) were not correlated with the FAB classification.

## Correlation of PLK4 with Survival in Childhood ALL Patients

At a median follow-up of 34.0 months, with a range of 2.0 to 50.0 months, 20 (21.1%) childhood ALL patients experienced disease progression or relapse, and 11 (11.6%) childhood ALL patients died.

PLK4 high was linked with shortened EFS in childhood ALL patients ($P = 0.020$); in addtition, 1-year, 2-year, and 3-year EFS rates were 93.6%, 91.4%, and 87.7%, respectively, in patients with low PLK4, while only 81.2%, 72.4%, and 67.1%, correspondingly, in childhood ALL patients with high PLK4(Fig. 3A). Besides, PLK4 high was not related to OS in childhood ALL patients ($P = 0.135$); moreover, 1-year, 2-year, and 3-year OS rates were 95.7%, 93.5%, and 93.5%, respectively, in patients with low PLK4, compared with 91.6%, 86.9%, and 83.4%, correspondingly, in childhood ALL patients with high PLK4 (Fig. 3B).

To further verify the association of PLK4 with EFS and OS in childhood ALL patients, the multivariate Cox's regression analyses using the enter method and backward-stepwise method were performed, respectively. In multivariate Cox's regression analysis using enter method, PLK4 high (vs. low) was independently linked with shortened EFS [hazard ratio (HR): 4.573, 95% CI: 1.112 to 18.809, $P = 0.035$]; also, in multivariate Cox's regression analysis using the backward-stepwise method, PLK4 high (vs. low) was also independently associated with reduced EFS (HR: 3.228, 95% CI: 1.080-9.652, $P = 0.036$) (Supplementary Table 2, Supplemental Digital Content 2, http://links.lww.com/JPHO/A547).

As to the association of PLK4 with OS, the multivariate Cox regression analyses using the enter method showed that PLK4 was not related to OS in childhood ALL patients (HR: 1.503, 95% CI: 0.204 to 11.071, $P = 0.689$)

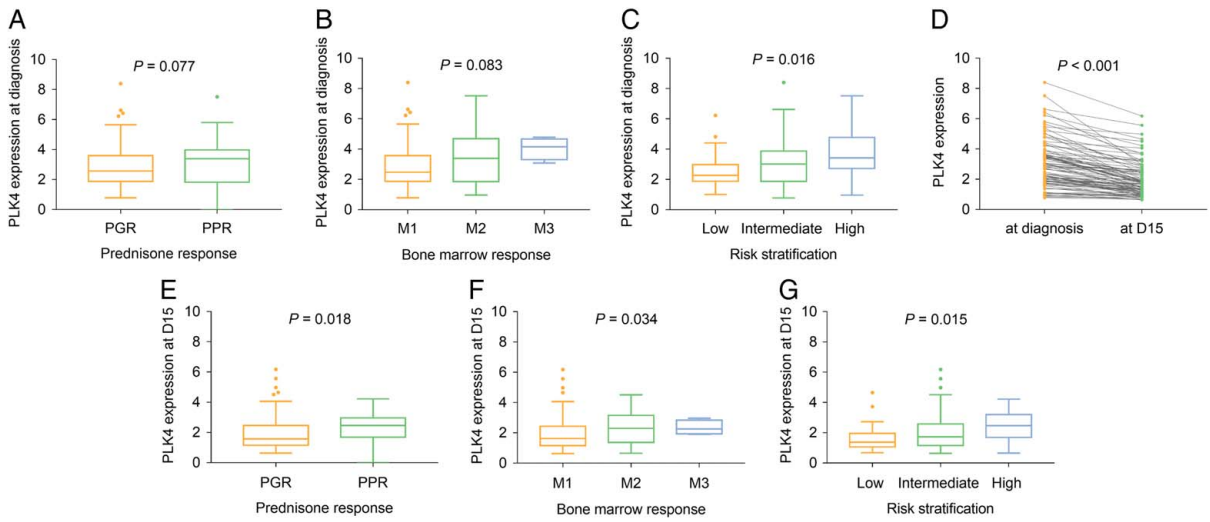


**FIGURE 2.** PLK4 correlated with treatment response and risk stratification in childhood ALL patients. Association of PLK4 at diagnosis with prednisone response (**A**), bone marrow response (**B**), CMA risk stratification, and (**C**) in childhood ALL patients. Change of PLK4 from diagnosis to D15 after treatment in childhood ALL patients (**D**). Correlation of PLK4 at D15 after treatment with prednisone response (**E**), bone marrow response (**F**), CMA risk stratification, and (**G**) in childhood ALL patients. ALL indicates acute lymphoblastic leukemia; CMA, Chinese Medical Association; PLK4, polo-like kinase 4. full color online

**TABLE 2.** Correlation of PLK4 Expression With Immunophenotype and FAB Classification

| Items | PLK4 at Diagnosis | | PLK4 at D15 | |
|---|---|---|---|---|
| | Median (IQR) | *P* | Median (IQR) | *P* |
| Immunophenotype | | 0.027 | | 0.033 |
| T-ALL | 4.330 (2.065-5.505) | | 2.460 (1.725-3.590) | |
| B-ALL | 2.705 (1.860-3.560) | | 1.705 (1.148-2.480) | |
| FAB classification | | 0.198 | | 0.246 |
| L1 | 2.570 (1.885-3.560) | | 1.725 (1.158-2.513) | |
| L2 | 3.240 (2.535-5.215) | | 1.900 (1.255-3.080) | |
| L3 | 1.910 (1.748-4.255) | | 1.655 (1.395-2.545) | |

B-ALL indicates B cell acute lymphoblastic leukemia; D15, day 15 after the initiation of induction therapy; FAB, French-American-British; IQR, interquartile range; PLK4, polo-like kinase 4; T-ALL, T cell acute lymphoblastic leukemia.

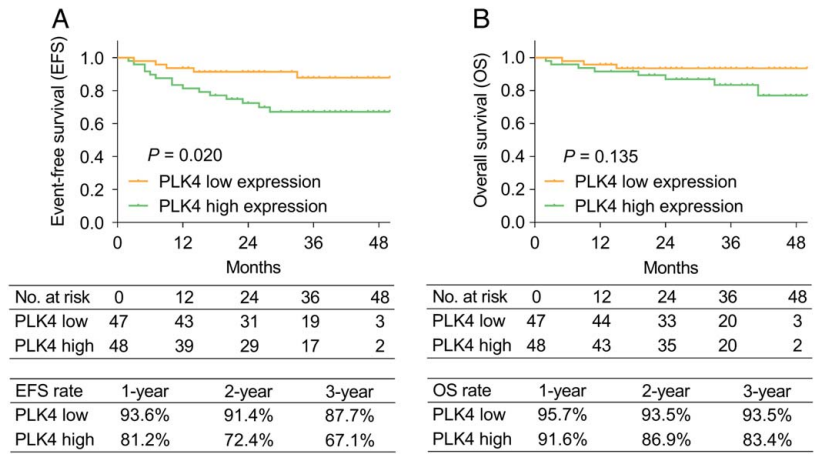


**FIGURE 3.** PLK4 high was related to shortened EFS in childhood ALL patients. Correlation of PLK4 with EFS (**A**) and OS (**B**) in childhood ALL patients. EFS indicates event-free survival; OS, overall survival; PLK4, polo-like kinase 4. full color online

(Supplementary Table 3, Supplemental Digital Content 3, http://links.lww.com/JPHO/A548).

## DISCUSSION

This study disclosed the following discoveries: (1) PLK4 was more elevated in childhood ALL patients than in controls. (2) PLK4 was more decreased at D15 after treatment compared with diagnosis in childhood ALL patients. (3) Increased PLK4 at D15 after treatment was associated with PPR, poor bone marrow response, and elevated CMA risk stratification. (4) PLK4 high was linked with shortened EFS in childhood ALL patients but not with OS.

PLK4, also known as staphylokinase, is a serine/threonine kinase belonging to the PLK family involved in DNA replication, cell cycle, and many other intracellular processes.[11,23,24] Recently, several studies have identified dysregulated expression of PLK4 in several malignant hematological diseases (including multiple myeloma, diffuse large B-cell lymphoma, etc.).[14–16] For instance, a previous study found that PLK4 was expressed abnormally and regulated centrosome formation in multiple myeloma patients.[16] Another study disclosed that PLK4 was increased in lymph node specimens from patients with diffuse large B-cell lymphoma than in patients with reactive lymphoid hyperplasia.[15] However, until now, the expression level of PLK4 in childhood ALL patients remains unclear. The current study found that PLK4 was more elevated in childhood ALL patients than in controls. The possible reason might be as follow: PLK4 promoted tumorigenesis and progression in childhood ALL patients; hence, it was upregulated in childhood ALL patients compared with controls.[12]

In addition to comparing PLK4 in childhood ALL patients and controls, this study also showed that PLK4 at D15 after treatment was decreased in childhood ALL patients than that at diagnosis; moreover, increased PLK4 at D15 after treatment was associated with PPR, poor bone marrow response, and elevated CMA risk stratification. The possible explanations were as follows: (1) PLK4 was mainly expressed in malignant lymphoid cells and extensively killed after treatment; consequently, PLK4 was correspondingly declined after treatment in childhood ALL patients.[13,25] (2) The previous studies disclosed that PLK4 interfered with the viability of bone marrow mesenchymal stem cells, and its impairment promoted the disease progression and drug resistance of childhood ALL patients.[24,26] Therefore, increased PLK4 at D15 after treatment was associated with PPR and poor bone marrow response in childhood ALL patients. (3) CMA risk stratification was determined by the number of risk factors (including prednisone response, bone marrow response, WBC, etc.).[22] Meanwhile, PLK4 was correlated with prednisone response and bone marrow response in this study. Taken together, PLK4 might reflect risk stratification of prognosis. Hence, the increased PLK4 at D15 was correlated with elevated CMA risk stratification in childhood ALL patients. Besides, this study also exhibited that both PLK4 at diagnosis and PLK4 at D15 after treatment were elevated in T-ALL patients than in B-ALL patients. This may be due to the following reasons: T-ALL cells usually had more severe malignant behaviors (including proliferative capacity and migration ability, etc.) than B-ALL cells.[27] Hence, PLK4 was more increased in T-ALL patients than in B-ALL patients.

The relationship between PLK4 with the prognosis of blood malignancies has been rarely reported, not to mention childhood ALL. This study collected survival data (progression, relapse, and death) and disclosed that PLK4 high was linked with shortened EFS in childhood ALL patients, whereas there was no correlation with the OS in childhood ALL patients. The probable reasons might be that: (1) It has been reported that PLK4 high lead to uncontrolled cell proliferation and cell cycle aberrations, which promoted the progression and relapse of ALL.[13] Thus, PLK4 high was correlated with shortened EFS in childhood ALL patients. (2) As mentioned above, increased PLK4 was associated with treatment response and CMA risk stratification; furthermore, the early treatment response could predict the survival of childhood ALL patients.[28] As a result, PLK4 high was related to shortened EFS in childhood ALL patients. (3) The relatively few deaths during the follow-up weakened the statistical power. Hence, PLK4 high was not associated with OS in childhood ALL patients.

Some limitations were identified in the current study. Firstly, this was a single-center study, which might result in selective bias; thus, it was necessary to conduct multiple-center studies in different regions to further validate the findings. Secondly, the number of patients was relatively small, which might weaken the statistical power, hence, the findings needed to be further validated in a prospective country-wide project with larger sample size.

Collectively, PLK4 may serve as a potential prognostic biomarker whose aberrant expression is correlated with PPR, poor bone marrow response, increased CMA risk stratification and shortened EFS in childhood ALL patients.

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