Expression of Wilms' Tumor Gene 1 and Its Clinical Significance in Children with Acute Lymphocytic Leukemia

Mengmeng Yin
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
Aiguo Liu
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
Ai Zhang
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
Yaqin Wang
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
Qun Hu (qunhu2013@163.com)
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
https://orcid.org/0000-0002-1508-2796

Research article

Keywords: Acute Lymphoblastic Leukemia, Children, Wilms' tumor 1 gene, Clinical significance

Posted Date: November 13th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-105168/v1

Abstract

Background: Wilms' Tumor Gene 1 *(WT1)* is a potential valuable parameter in prognosis of childhood acute lymphoblastic leukemia (ALL). However, studies on prevalence of *WT1* and its correlation to clinical features and prognosis in pediatric patients were not well done. In this study we attempted to identify the correlation between *WT1* and childhood ALL.

Methods: The expression levels of *WT1 in bone marrow cells of 188* children diagnosed with ALL from 2015 to 2018 were detected using real-time quantitative polymerase chain reaction (RQ-PCR). The relationship between expression levels of *WT1* and patients' characteristics, remission status (complete remission/relapse), fusion genes and prognosis of childhood ALL were analyzed and revealed.

Results: 1. 147 (78.2%) cases had positive *WT1* expression, and the average level was 1.76 (0.3, 6.03) %. 2. The CR and relapse rates of ALL children with positive *WT1* were not significantly different from those of *WT1* negative group, respectively (87.76% *vs* 82.93%, *P*=0.42 and 14.29% *vs* 17.1%, *P*=0.658). 3. The *WT1* expression level in patients at CR was significantly lower than when at diagnosis (*P*<0.001) and the expression of *WT1* increased obviously after induction therapy in 21 patients who relapsed (*P*=0.003) .4. The *WT1* expression was related to lymphadenectasis (*P*=0.004) and immunophenotyping (*P*=0.009), but not to fusion genes (*P*=0.912).

Conclusion: The *WT1 i*n ALL children can be employed as an independent tool to evaluate the prognosis and curative effect of the disease.

1. Background

Wilms' tumor gene 1 (*WT1*) located on human chromosome 11p13 was isolated from nephroblastoma in children in 1990 [1]. As a transcription factor encoded by tumor suppressor genes that can regulate cell growth, it is closely related to the occurrence and development of Wilms' tumors. The *WT1* encodes a zinc finger transcription factor. Posttranscriptional mRNA modification and the existence of several possible transcriptional initiation sites produce many different *WT1* protein isomers (at least 32), which are located in specific subcellular and subnuclear regions and have different functions, some of which are partially overlapping. *WT1* modulates the transcription of various target genes and participates in the processing of posttranscriptional mRNA. It has bidirectional transcriptional regulation and participates in the regulation of hematopoietic transcription [2]. *WT1* plays a major carcinogenic role in malignant hematological tumors, inhibiting the function of transcription factors and hindering the normal proliferation and differentiation of hematopoietic stem cells. At the same time, many factors can lead to mutation of *WT1*, rendering the *WT1* protein unable to regulate specific DNA and thus promoting the growth of tumor cells [3].

There is a significant correlation between the expression of *WT1* and the poor prognosis of malignant tumors in the human hematopoietic system. Although *WT1* is not specific to acute malignant hematological diseases, its continuous expression can be detected in almost all leukemia cells. Several

studies have demonstrated that *WT1* was over-expressed in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and chronic myeloid leukemia (CML), and it was highly expressed in more than 80% of ALL patients in bone marrow (BM) cells [4]. Therefore, *WT1* was considered to be a new "pan-leukemia" gene that involved in the proliferation and differentiation of leukemia cells. The quantitative assessment of *WT1 in acute leukemia could represent a universal molecular marker of malignant hematopoiesis and was suggested to* monitor minimal residual disease (MRD) and to predict the progression and prognosis of disease [5].

The biological functions of *WT1 in childhood ALL are not understood clearly and completely, but it has been suggested that WT1* can impact the pathogenesis of leukemia during cellular proliferation and differentiation. In this paper, we analyzed *WT1* expression level in ALL children to reveal the predictive role of *WT1* as an MRD marker in this group of patients.

2. Methods

2.1 Patients, treatment and follow-up

A total of 188 children newly diagnosed with ALL between 2015 and 2018 in our hospital were enrolled. We collected all the patients' characteristics and measured the expression levels of *WT1* at diagnosis and during treatment. All patients were followed up as of October 2020. A cohort of the 188 patients with ALL were consisted of 118 males (62.8%) and 70 females (37.2%), with a median age of 3.2 years. In accordance with WHO leukemia diagnostic criteria，all the patients were diagnosed by morphology combined with immunology, cytogenetics and molecular biology (MICM) of bone marrow cells. Patients' treatment was given based on CCCG (Chinese Children Cancer Group) –ALL 2015 regimen.

MRD testing is used to detect the proportion of remaining leukemia cells in bone marrow at the end of induction chemotherapy (IC). A cut-off point for MRD testing by flow cytometry (FCM) is defined at the level of 0.01%. Thus, MRD lower than 0.01% means complete remission (CR).

2.2 Morphology

Morphologic criteria were established based on French-American-British (FAB), WHO 2004 or WHO 2008 classification.

2.3 Immunophenotyping

Immunophenotyping was conducted by FCM, using monoclonal antibodies to determine lineage-specific antigens, and the panel of tests included at least the following: CD10, CD19, CD20, CD23, cCD79a, SmIg for B-ALL or CD1, CD2, CD3, CD4, CD5, CD7, CD8, TdT for T-ALL.

2.4 Cytogenetics

Chromosome banding of BM samples was performed using R-banding or G-banding technique with short-term cultures. The reverse-transcription polymerase chain reaction (RT-PCR) or fluorescence *in situ* hybridization (FISH) was performed to detect common fusion genes such as *TEL-AML1*, *E2A* (also called *TCF3*)-*PBX1*, *BCR-ABL*, and *MLL* (also called *KMT2A*)-related rearrangements.

The real-time quantitative PCR (RQ-PCR) was applied to measure the *WT1* expression level in BM samples from the 188 ALL children. We cloned both *WT1* and *ABL* genes for creating a standard curve and calculated copy numbers of *WT1* gene in these patients.

1. RNA extraction: 4ml bone marrow was collected from the patients and put into 2% EDTA for anticoagulation. Mononuclear cells were separated by Ficoll lymphocyte isolate solution. After washing the cells with PBS, 1 mL Trizol (invitrogen) was added, blown evenly and stored in -80℃ until the RNA was extracted. The extraction of total RNA was carried out according to the instructions of Trizol reagent. The A 260nm /A 280nm ratio was determined by ultraviolet spectrophotometer to identify the purity and quantity of RNA and then frozen under -80℃.The extracted RNA were mixed with RNase-free water and dissolved, and the 15 mL treatment fluid was used as the PCR reaction template.
2. Real-time quantitative PCR: PCR method was used to detect *WT1* gene mRNA. The instrument was a ABI7500 fluorescence detector, the reagent was derived from the tumor-related *WT1* gene detection kit produced by Shanghai Yuanqi Biotechnology Co. Ltd. The steps were operated according to the operating instructions of the kit. The reaction conditions were 42℃ for 30 min, 94℃ for 5 min, 94℃ denaturation for 15 s, 60℃ annealing for 30 cycles. The reaction system was 25μL. The fluorescence signal was collected at 60℃ in the second step of PCR cycle.
3. Calculation of *WT1* gene expression level: Four successful serial dilutions (1×10^6, 1×10^5, 1×10^4 and 1×10^3copies) of each gene were prepared. The copy number of Abelson proto-oncogene (*ABL*) and *WT1* gene were calculated according to the standard curve by *ABL* expression as the internal reference. When 1×10^2 copies≤ *WT1* RNA detection concentration≤1×10^7 copies and 1×10^2 copies≤ *ABL* RNA detection concentration≤1×10^7 copies, the *WT1* expression level = (*WT1* copy numbers/ *ABL* copy numbers)× 100%.

2.5 Statistical analyses

Statistical analysis was carried out using SPSS software version 23.0 (IBM Corporation, Armonk, USA), and the count data were expressed as percentage or constituent ratios. Parametric and nonparametric tests including Mann–Whitney, Chi-square test，Student's t test and ANOVA were applied according to data distribution. Experiment data were described in the form of mean ± standard deviation or median (25th, 75th) in accordance with data distribution in our study and $P < 0.05$ was considered statistically significant.

3. Results

3.1 *WT1* expression in ALL children

Among the 188 children with ALL, 147 were tested positive for *WT1*, 41 were negative, as the positive rate was 78.2%. The average expression level was 1.76 (0.3–6.03) % in *WT1* positive group. We compared *WT1* expression level in different patients' characteristics and found that it was only related to lymphadenectasis (*P*=0.004) (Table 1).

3.2 Association between *WT1* and immunophenotyping

In our study cohort, there were 22 children with T-ALL, of whom 17 (77.27%) with *WT1* positive, and 78.31% (130/166) of B-ALL children were *WT1* positive. There was no significant difference in the positive rate of *WT1* between the two groups (77.27% *vs* 78.31%, *P* =0.912), but expression level of *WT1 in* T-ALL children [7.4(1.03, 18.91) %] was obviously higher than that in B-ALL children [1.61(0.23, 4.84) %] (*P*=0.009).

3.3 Association between *WT1* and fusion genes

In our group, a total of 62 cases had fusion genes including *TEL-AML1, BCR-ABL1, E2A-PBX1, MLL* rearrangements and *SIL-TAL1*. 88.9% (32/36) of children with *TEL-AML1* were positive for *WT1* gene. The incidence of *WT1in BCR-ABL1, E2A-PBX1* and *MLL* rearrangements group was 87.5% (7/8), 88.9% (8/9) and 85.7% (6/7), respectively. The *WT1* was positive in both of the children with *SIL-TAL1*. There were 55 cases carried *WT1* and other fusion genes simultaneously, the other 7 cases with single fusion gene. There was no significant difference in expression of *WT1* among ALL children carried the above five fusion genes when at diagnosis and at CR, respectively (*P* = 0.415, *P* = 0.861) (Table 2).

3.4 Comparison of CR and relapse rates in different groups

There were 129 children achieved CR in the *WT1* positive group and 34 children achieved CR in the *WT1* negative group. The CR rates of *WT1* gene-positive group and gene-negative group after induction chemotherapy were 87.76% and 82.93%, respectively (*P*=0.42). By October 2020, there were 21 children relapsed in the positive group and 7 children relapsed in the negative group, with relapse rates of 14.29% and 17.1%, respectively (*P* = 0.658). The CR and relapse rates of two groups both had no significant difference.

3.5 Expression of *WT1* during the course of disease

The expression of *WT1 in* the 147 positively expressed ALL children was monitored after chemotherapy. Among the 129 children with CR after IC, the expression level of *WT1* decreased in 109 cases (84.5%, 109/129) and increased in 20 cases (15.5%, 20/129). The expression of *WT1* between at diagnosis and CR had a significant difference (*P* <0.001). The patients at CR showed a significantly lower expression of *WT1* than at diagnosis. However, *WT1* expression of 18 children who didn't get CR had no significant difference between at diagnosis and after IC (Table 3, Figure 1). Finally, 14 children (12.84%, 14/109) relapsed in the 109 children with decreased *WT1* expression and 4 relapsed in 20 ones with increased

WT1 expression (20%, 4/20).There was no significant difference (12.84% *vs* 20%, *P*=0.47), meaning risk of relapse is not correlated on initial WT expression levels.

3.6 Changes of *WT1* expression in ALL children after relapse

Up to the follow-up time, there were 21 children relapsed in *WT1* gene-positive group finally, and 18 of whom achieved CR after IC (Table 4). Among the 21 relapsed patients with positive *WT1*, their *WT1* expression after relapse [2.28±0.25,1.69±%] showed higher than that after IC [0.33±0.045,0.66±%] (*P* =0.003) (Figure 2).

4. Discussion

The innovation of chemotherapeutic drugs and allogeneic hematopoietic stem cell transplantation (allo-HSCT) can significantly improve the remission and survival rates of children with ALL, but the relapse after tumor remission is still a difficult problem [6]. The results of MRD at the end of induction phase of chemotherapy in BM testing are used to reclassify risk levels of ALL patients and guide changes in treatment according to their future risk of relapse. The timely monitoring of MRD in ALL children can provide them individualized and intensified treatment [7]. An enormous amount of literature suggested that high expression of *WT1* before and after allo-HSCT is a poor prognosis factor. It is of clinical practical value to use *WT1* as a transplant recommendation indicator for ALL patients and as a marker to monitor MRD dynamically. Specific marker genes of ALL, such as *TEL/AML1*, *BCR/ABL1* and *E2A/PBX1* can be monitored. Although the altered *WT1* expression seems inherent in leukemia cells, the molecular mechanisms by which *WT1* contribute to leukemogenesis have not yet been fully elucidated. The role of *WT1* dosage in steady-state and pathologic hematopoiesis needs to be investigated by further studies [8]. The literature reported the positive rate of *WT1 in* ALL children was more than 80%, and in our study the positive rate was 78.2% which was consistent with that reported in the literature. By FCM, BM samples can give a sufficient sensitivity in the vast majority of patients. *WT1* expression can be used as a panleukemic marker and is also a good marker for ALL patients with no other molecular or cytogenetic abnormalities [9]. Since the majority of ALL children expressed very high values of *WT1* at diagnosis, the detection of *WT1* can be considered a tool of value to monitor the persistence of the disease after chemotherapy or BM transplantation or during the treatment [10].

We collected all patient characteristics including age, sex, chromosome and so on to analyze whether there was an association between *WT1* and them. Among all characteristics, we only found lymphadenectasis was related to *WT1 in childhood ALL. The phenomenon of lymphadenectasis happened in ALL children without WT1* expression was more common than those with *WT1* (53.66% *vs* 29.25%, *P*=0.004).

T-ALL is biologically distinct from B-ALL and differences in response to chemotherapy have been observed. The prognosis of children with T-ALL is worse than those with B-ALL, which is caused by different fusion transcripts and prognostic factors had been investigated extensively. To determine whether immunophenotyping of ALL can impact *WT1* expression, we compared expression of *WT1 in T-*

ALL and B-ALL children. Later findings demonstrated that WT1 expression upregulated in T-ALL due to the continuous activation of NF-KB pathway, which meanwhile has recognized as a vital role in the mechanism of refractory relapse in T-ALL children [11]. In our present study, incidence of *WT1 in* T-ALL and B-ALL children did not differ, but expression level of *WT1 in* T-ALL children was significantly higher than that in B-ALL children. Considering this result, we can put forward a hypothesis that *WT1* involved in the progression and relapse of T-ALL and contributed to diverse prognosis in different immunophenotyping of ALL. Bordin et al. reported that loss-of-function mutations and deletions in *WT1* emerged in approximately 10% of T-ALL children. Clinically, *WT1* mutations are enriched in relapsed series and play a critical role in the response to DNA damage in T-cell leukemia. Some studies based on *WT1* positively affecting the expression of the X-linked inhibitor of apoptosis protein (XIAP) to restore sensitivity to γ-radiation therapy supported the view that anti-XIAP targeted therapies might be beneficial to treatment of T-ALL patients with *WT1* [12].

We carried out a study which compared the *WT1* expression between individuals positive for fusion genes including *TEL-AML1, BCR-ABL1, E2A-PBX1, MLL* rearrangements and *SIL-TAL1*. Through the statistical data, we found *WT1* and other fusion genes coexisting in ALL children were common. This was also in accordance with the theory that *WT1* presented in majority of ALL children. The various fusion transcripts have been confirmed to be highly predictive of clinical outcomes and play different roles in the prognosis of ALL. For example, the Philadelphia chromosome-positive (Ph+) ALL is defined by the t (9; 22) (q34; q11) translocation that forms the fusion gene *BCR-ABL1*, which produces an abnormal protein, contributing to the sustaining activation of tyrosine kinase. It predicts poor clinical outcomes due to its resistance to chemotherapy and high relapse risk. Several researches suggested that *BCR-ABL1* fusion protein may prompt *WT1* expression by activating some signaling pathways, and decrease the sensitivity to chemotherapeutic drugs [13]. Svensson et al. presented that *BCR-ABL1* fusion protein could increase the expression of *WT1* mRNA and protein via the phosphatidylinositol-3 kinase (PI3K)-Akt pathway. Their experiment results indicated that *WT1* expression was induced by oncogenic signaling from *BCR-ABL1* and that *WT1* contributed to resistance against apoptosis induced by imatinib[14]. The t (12; 21) (p13;q22) producing *TEL-AML1* is the most common reciprocal translocation in childhood B-ALL. It is associated with favorable prognosis following conventional therapeutic strategies. But the interaction between *TEL-AML1* fusion protein and *WT1* is not certain. Similarly, the role of *WT1 in other* fusion transcripts is still unclear. Qin et al. reported that *WT1* expression was obviously different among diverse cytogenetics groups (*TEL-AML1, E2A-PBX1* and *MLL* rearrangements) [15]. However, there was no difference in expression level of *WT1* among these five different fusion transcripts groups when at diagnosis and CR in our study. The discrepancy might be caused by the small number of patients and different detection methods.

Among the 129 ALL children having achieved CR, 109 (84.5%, 109/129) got their *WT1* expression descended or negative, 20 (15.5%, 20/129) got elevated in *WT1* expression conversely. Among the 18 relapsed children with *WT1*, the expression of *WT1* decreased in 14 cases and increased in 4 cases after CR. Moreover, the expression levels of *WT1* after relapse were all higher than those after IC. These results were in agreement with literature data [16].

The clinical value of altered *WT1* expression in response to chemotherapy, guiding therapeutic intervention and predicting relapse is still not determined, and the conclusions of different reports are inconsistent. Some researches have found that *WT1* was over-expressed in both peripheral blood (PB) and BM cells of the majority of ALL children, at a level that enabled a significantly more sensitive evaluation of residual disease than standard immunophenotypic and morphological analyses. Meantime, *WT1* could have abnormally high expression in malignant cells compared with normal controls, which might be a candidate for MRD monitoring. Several studies confirmed that the expression of *WT1 in ALL could cause resistance of cells to* differentiation and apoptosis, and it might lead to poor clinical outcomes. For example, it has been reported that expression level of WT1 after T-ALL transplantation was significantly negatively correlated with relapse risk [17]. Based on the information contained in other studies, they thought the expression of *WT1 in* ALL children is so variable that cannot be accurately calculated as MRD during treatment. Because of the inaccuracy of the assessment, *WT1* cannot evaluate the prognosis of ALL [2]. While Inoue et al compared CR rate of ALL children in *WT1* gene-positive group and gene-negative group, it revealed a significant difference [18]. However, Chiusa et al. reported that no correlation was observed between *WT1* and therapeutic effect of leukemia [19]. In our group, the CR rate of *WT1* gene-positive patients (87.76%) and gene-negative patients (82.93%) did not statistically differ. The interaction between therapy response and *WT1* is still required more studies to be established.

The presence of MRD following therapy for ALL has been demonstrated to be a crucial predictor of relapse in many current studies. MRD typically detected by FCM is on the base of leukemic cells expressing combinations of antigens that are different from those exist in normal BM cells. Particularly in ALL children, the value of MRD must be balanced against other well-established prognostic indexes, but its correlation with other prognostic indexes has not been fully assessed [20]. In this analysis, we found 14.29% (21/147) in patients with *WT1* and 17.1% (7/41) without *WT1* relapsed after therapy. The relapse rate in these two groups was not significantly altered. The small number of cases and short follow-up time may lead to the lack of significant difference between the two groups. Some studies have found that there was a horizontal relationship between expression level of *WT1* and progression of ALL, which would increase, decrease and then increase corresponding to the stage of disease diagnosis, remission and relapse, respectively. A similar trend was observed in our study. Significant decrease in *WT1* expression was observed in 129 patients after CR compared with those at diagnosis. That means it can indict the patients' therapeutic effect. Meanwhile, a significant increase in *WT1* expression was witnessed in ALL patients after relapse. 21 relapsed children got their *WT1* over-expressed which accorded with some literature documents [5]. From the results obtained so far, it seems that prognosis in ALL children is inversely associated with the expression of *WT1*.

There are several limitations in our study. First, as there were not enough patients, especially for some fusion genes group, patients are so few that could not be compared comprehensively. Second, a little shorter follow-up time may leave out some relapsed patients.

5. Conclusion

From the above discussion, the conclusion can be reached that *WT1* can be used as a suitable marker to monitor MRD, assess response to chemotherapy and predict relapse in the majority of ALL children. Adding to the patients carrying a fusion gene, we can also use RQ-PCR technology to detect *WT1* to assess disease risk. By employing such methods, we can determine the effect of induction chemotherapy and state in disease after therapy. Collectively, our study results support the use of *WT1* as a marker to monitor childhood ALL progression dynamically.

Abbreviations

WT1: Wilms' Tumor Gene 1

ALL: acute lymphoblastic leukemia

RT-PCR: reverse-transcription polymerase chain reaction

RQ-PCR: real-time quantitative polymerase chain reaction

MRD: minimal residual disease

AML: acute myeloid leukemia

CML: chronic myeloid leukemia

BM: bone marrow

MICM: morphology, immunology, cytogenetics and molecular biology

CCCG: Chinese Children Cancer Group

FAB: French-American-British

IC: induction chemotherapy

FISH: fluorescence *in situ* hybridization

FCM: flow cytometry

ABL: Abelson proto-oncogene

PB: peripheral blood

Declarations

Acknowledgements

Not applicable

Authors' contributions

Conception and design: QH and MM. Data analysis and interpretation: MM. Writing, review, and revision of the manuscript: QH and MM. Collection and assembly of data: all authors. The authors read and approved the final manuscript.

Funding

No funding.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. This study was approved by the Ethical review committee of Tongji Medical College, Huazhong University of Science and Technology. The reference number: S207. All written informed consent was obtained from a parent or guardian for participants under 16 years old.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Lee, S.B. and D.A. Haber, Wilms tumor and the WT1 gene. Exp Cell Res, 2001. 264(1): p. 74-99.
2. Boublikova, L., et al., Wilms' tumor gene 1 (WT1) expression in childhood acute lymphoblastic leukemia: a wide range of WT1 expression levels, its impact on prognosis and minimal residual disease monitoring. Leukemia,2006. 20(2): p. 254-63.
3. Toska, E. and S.G. Roberts, Mechanisms of transcriptional regulation by WT1 (Wilms' tumour 1). Biochem J, 2014. 461(1): p. 15-32.
4. Wang, X.R., et al., Overexpressed WT1 exhibits a specific immunophenotype in intermediate and poor cytogenetic risk acute myeloid leukemia. Ann Hematol, 2020. 99(2): p. 215-221.
5. Dou, C.Y., et al., [Relationship between Quantitative Monitoring of WT1 Gene and Prognosis in Patients with Acute Leukemia]. Zhongguo Shi Yan Xue Ye Xue Za Zhi, 2018. 26(6): p. 1632-1636.

6. Jiang, B.Q., et al., [The effect of WT1 expression on the prognosis of allogeneic hematopoietic stem cell transplantation in acute leukemia]. Zhonghua Xue Ye Xue Za Zhi, 2018. 39(12): p. 989-993.
7. Minimal Residual Disease Evaluation in Childhood Acute Lymphoblastic Leukemia: A Clinical Evidence Review. Ont Health Technol Assess Ser, 2016. 16(7): p. 1-52.
8. Pronier, E., et al., Genetic and epigenetic evolution as a contributor to WT1-mutant leukemogenesis. Blood, 2018. 132(12): p. 1265-1278.
9. Gupta, S., et al., Flow-cytometric vs. -morphologic assessment of remission in childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group (COG). Leukemia, 2018. 32(6): p. 1370-1379.
10. Zhang, R., et al., Comparison of minimal residual disease (MRD) monitoring by WT1 quantification between childhood acute myeloid leukemia and acute lymphoblastic leukemia. Eur Rev Med Pharmacol Sci, 2015. 19(14): p. 2679-88.
11. Gao, H.L., et al., [Expression Levels of HES1, C-MYC and NF-kB in Peripheral Blood of Patients with T Cell Acute Lymphoblastic Leukemia and Their Significance]. Zhongguo Shi Yan Xue Ye Xue Za Zhi, 2019. 27(5): p. 1449-1454.
12. Bordin, F., et al., WT1 loss attenuates the TP53-induced DNA damage response in T-cell acute lymphoblastic leukemia. Haematologica, 2018. 103(2): p. 266-277.
13. Qin, Y.Z., et al., The prognostic significance of Wilms' tumor gene 1 (WT1) expression at diagnosis in adults with Ph-negative B cell precursor acute lymphoblastic leukemia. Ann Hematol, 2019. 98(11): p. 2551-2559.
14. Svensson, E., et al., Deregulation of the Wilms' tumour gene 1 protein (WT1) by BCR/ABL1 mediates resistance to imatinib in human leukaemia cells. Leukemia, 2007. 21(12): p. 2485-94.
15. Qin, Y.Z., et al., [An interlaboratory comparison study on the detection of RUNX1-RUNX1T1 fusion transcript levels and WT1 transcript levels]. Zhonghua Xue Ye Xue Za Zhi, 2019. 40(11): p. 889-894.
16. Owen, C., J. Fitzgibbon and P. Paschka, The clinical relevance of Wilms Tumour 1 (WT1) gene mutations in acute leukaemia. Hematol Oncol, 2010. 28(1): p. 13-9.
17. Woehlecke, C., et al., Prognostic impact of WT1 expression prior to hematopoietic stem cell transplantation in children with malignant hematological diseases. J Cancer Res Clin Oncol, 2015. 141(3): p. 523-9.
18. Inoue, K., et al., WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. Blood,1994. 84(9): p. 3071-9.
19. Chiusa, L., et al., Prognostic value of quantitative analysis of WT1 gene transcripts in adult acute lymphoblastic leukemia. Haematologica, 2006. 91(2): p. 270-1.
20. Hagag, A.A., et al., Prognostic Impact of WT-1 Gene Expression in Egyptian Children with Acute Lymphoblastic Leukemia. Mediterr J Hematol Infect Dis, 2016. 8(1): p. e2016008.

Tables

Table 1. Patient characteristics

Characteristic	*WT1* (+)（*n*=147）	*WT1* (-)（*n*=41）	*P*
Sex			0.404
Male	93	23	
Female	54	18	
Age, years	5.9（3.3,8）	5.1（3,6.2）	0.191
Immunophenotyping			0.912
T-ALL	17	5	
B-ALL	130	36	
Karyotype			0.995
Hyperdiploid(<46 chromosomes)	37	10	
Diploid(=46 chromosomes)	103	29	
Hypodiploid(>46 chromosomes)	7	2	
Liver/splenomegaly (subcostal >5 cm)	21	126	0.732
lymphadenectasis	43	22	0.004*
CR after IC	129	34	0.42
Relapse	21	7	0.658
Leukocyte count（×10^9/L	31.07（4.05,27.94）	18.62（2.8,18.4）	0.242
Hemoglobin count, g/L	77.52±23.6	74.69±24.89	0.503
Platelet count, ×10^9/L	93.24(31,135)	110.7(36,129)	0.652
Myeloid juvenile cell（%	41.64（10,71）	40.02（8.5,69.5）	0.765
Serum LDH level, IU/L	572.3±133.6	472.93±124.9	0.23

Counting data were expressed by number of people, and measurement data were described in the form of mean ± standard deviation or median (25th, 75th) were in accordance with data distribution.

ALL: acute lymphoblastic leukemia; CR: complete remission; IC: induction chemotherapy; LDH, lactate dehydrogenase.

*P<0.05: statistically significant.

Table 2. Changes in the expression level of *WT1 in fusion genes*

Fusion gene	*n*	Diagnosis (%)	After CR (%)
[*TEL-AML1*]	32	2.09 (1.03,4.08)	0.23 (0.08,0.68)
[*BCR-ABL1*]	7	2.4 (1.46,8.3)	0.31 (0.02,1.02)
[*E2A-PBX1*]	8	4.53±2.48	0.66±0.58
[*MLL*]	6	4.43±3.80	0.67±0.43
[*SIL-TAL1*]	2	11.64 (0.72,22.55)	3.08 (0.05,6.1)
P		0.415	0.861

CR: complete remission

Table 3. *WT1* expression level after IC

Course of disease	CR after IC (%) (*n*=129)	Not CR after IC (%) (*n*=18)
Diagnosis	1.68(0.28,5.8)	4.48(0.78,5.03)
After IC	0.18(0.06,0.62)	1.91(0.77,1.8)
P	<0.001*	0.24

CR: complete remission; IC: induction chemotherapy.

*P<0.05: statistically significant.

Table 4. Dynamic changes in expression of *WT1 in relapsed patients*

Case	Diagnosis (%)	After IC (%)	Relapse (%)
1	2.4	0.02	0.14
2	0.91	0.19	0.39
3	1.2	0.56	1.94
4	0.01	0	0.2
5	0.14	0.29	0.19
6	0.46	0.15	22.82
7	2.08	0.18	0.91
8	0.17	0.02	0.3
9	0.81	0.67	1.43
10	0.99	0	0.06
11	0.75	0.3	0.45
12	0.86	0.15	2.7
13	0.05	0.68	0.5
14	0.9	1.12	4.41
15	1.76	0.15	0.3
16	0.38	0.02	1.04
17	0.06	0.64	7.89
18	1.39	0.07	0.31
19	1.55	0.16	1.37
20	4.7	0.9	0.4
21	0.3	0.67	0.06

ALL: acute lymphoblastic leukemia; IC: induction chemotherapy

Figures

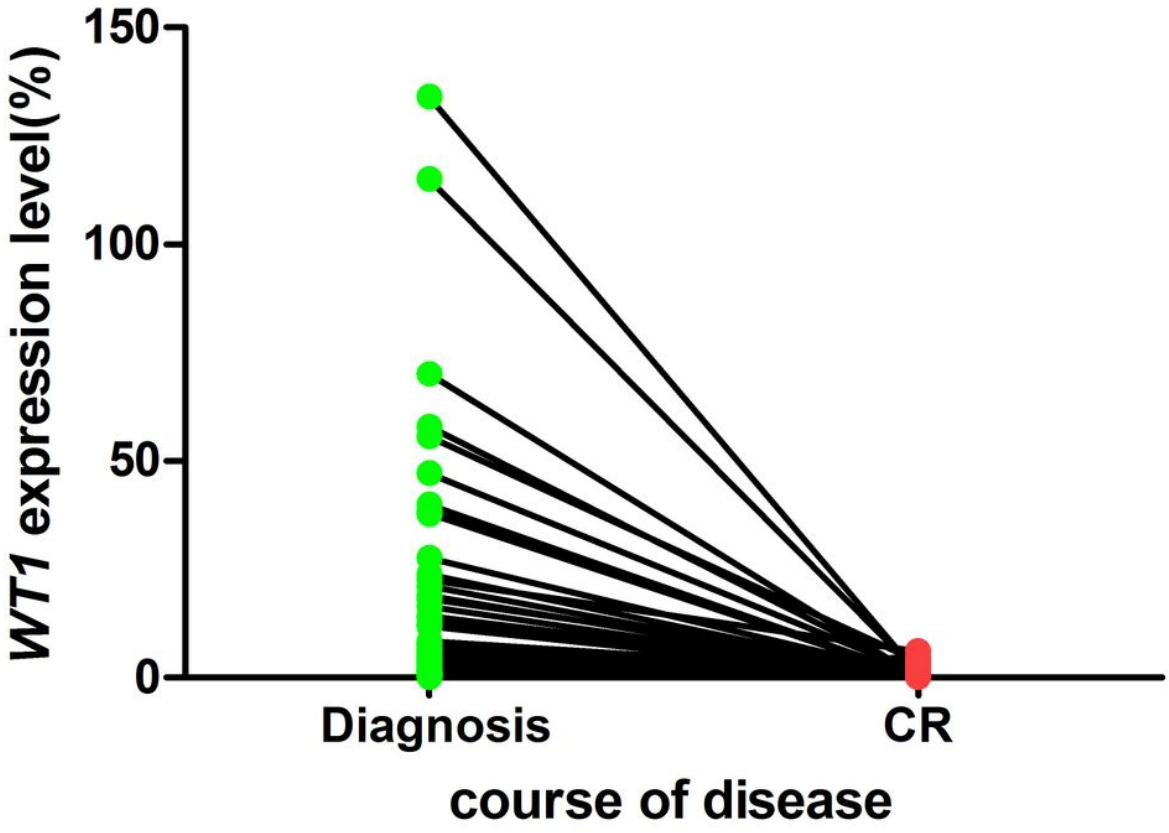

Figure 1

Changes of WT1 expression during course of ALL disease The red and green graphics show the expression level of WT1 at diagnosis and CR in ALL children, respectively. There was a significant difference between WT1 expression at diagnosis and when at CR ($P < 0.001$). The patients achieved CR showed a significantly lower expression of WT1 than at diagnosis.

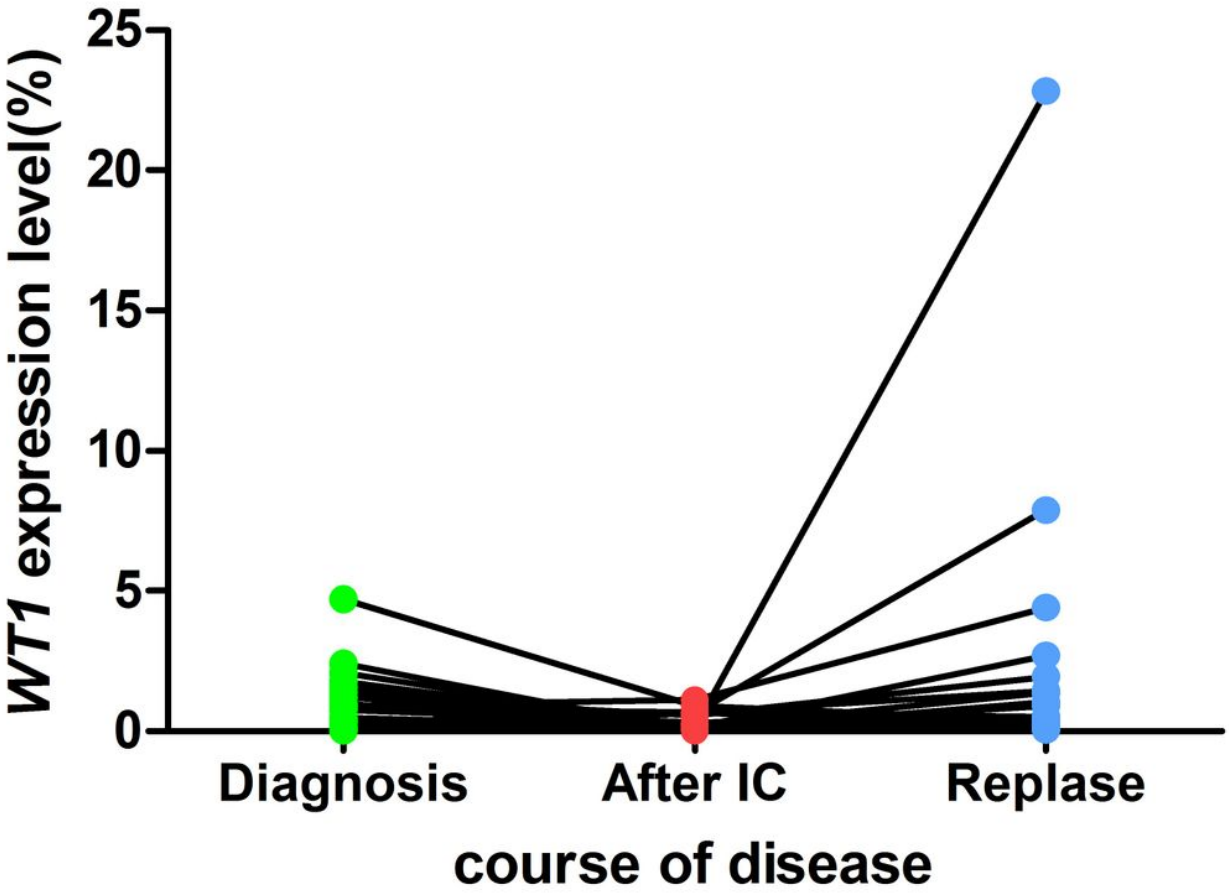

Figure 2

Changes in WT1 expression in ALL children after relapse The red, green and blue graphics show the WT1 expression level of relapsed patients at diagnosis, CR and after relapse to be compared, respectively. A change trend can be observed from this figure. There was a significant difference between WT1 expression at CR and when after relapse (P =0.003). For 21 relapsed patients with WT1, their expression of WT1 after relapse showed higher than that after induction chemotherapy.