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Application of minimal residual disease monitoring in pediatric patients with acute lymphoblastic leukemia

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Abstract. – Acute lymphoblastic leukemia (ALL) is a malignant neoplastic disease characterized by abnormal hyperplasia of immature lymphatic cells and has become the most common tumor in children. Although the efficacy of acute lymphoblastic leukemia in children was significantly increased with the adjustment of chemotherapy regimen, there were still a few patients who failed in treatment. The main reasons were relapse and drug resistance. Minimal residual disease (MRD) refers to a state in which there remain traces of leukemia cells that could not be detected using morphological methods in leukemia patients who are in complete remission after receiving the induction chemotherapy or bone marrow transplantation, which is considered to be the main cause of recurrence. The most commonly used methods for detection of MRD include flow cytometry (FCM), real-time quantitative polymerase chain reaction (RQ-PCR) and next-generation sequencing (NGS). MRD evaluation plays an important role in evaluating prognosis, predicting recurrence, guiding risk stratify and individualized therapy for children with ALL. In this paper, we reviewed the progresses in major detection methods for MRD that have been made in the clinical application of pediatric ALL.

Key Words:
Acute lymphoblastic leukemia, Minimal residual disease, Flow cytometry, Real-time quantitative polymerase chain reaction, Next-generation sequencing.

Introduction

Pediatric acute lymphoblastic leukemia (ALL) is a malignant neoplastic disease characterized by abnormal hyperplasia of immature lymphatic cells, which is of the great heterogeneity between the biological features and clinical prognosis. ALL, as the most common malignant tumor in children, has exhibited an increasing tendency on a year-by-year basis [1]. According to the Shanghai CDC (Shanghai Center for Disease Control and Prevention) Cancer Bulletin system, 4.7 out of every 100,000 children under the age of 15 have leukemia, according to which it is estimated that there are about 14000 new children with leukemia in China each year. As a result of chemotherapy regimen adjustment and hematopoietic stem cell transplantation (HCT) , the effect of acute lymphoblastic leukemia is improved, and the 5-year event-free survival (EFS) rate has over 80%; there are still about 10 to 15% of children suffering the recurrence that has been considered as a major obstacle for pediatric ALL patients enjoying a long-term survival, a major cause for death and a large problem in the treatment of leukemia[2,3]. Many researches[4,5] have confirmed that recurrence is mainly caused by the existence of minimal residual disease (MRD) in leukemia. MRD refers to a state in which there remain traces of leukemia cells that could not be detected using morphological methods in leukemia patients who are in complete remission after receiving the induction chemotherapy or bone marrow transplantation, which is believed to be the root cause of recurrence. Thus, a method with a high sensitivity, specificity, stability and low cost but great convenience should be developed to dynamically monitor the MRD in pediatric ALL patients, which is of great significance for assessing the response to treatment in an early stage, conducting the risk-based grouping, evaluating the prognosis and guiding the treatment for patients in recurrence and the individualized treatment.

Detection Method of MRD

In recent years, various methods have been used to detect the MRD; the most frequently employed

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methods include multi-parameter flow cytometry (MPFC), Real-time quantitative polymerase chain reaction (PCR) and next-generation sequencing (NGS); the major features of these methods have been summarized in Table I[6,7,22-24].

FCM

The basic principle to detect ALL-MRD by FCM is that multi-parameter quantitative analysis is carried out to identify the abnormal immunephenotypes on the surface of leukemia cells, that is, the leukemia associated immunophenotypes (LAIPs); this method, with a sensitivity of 10^{-4}-10^{5}, can identify the leukemia cells from the normal or regenerative bone marrow cells[6]. EuroFlow-based FCM (≥ 8 colors) appear to be able to achieve improved sensitivities (as low as 10^{-6}) and thus may further improve on the utility of MFC in the risk assessment of ALL [7]. The LAIPs can be divided into 3 categories: the first category is the immunophenotype of naïve T cell. Common immune markers for detection of T-ALL MRD include CD2, CD3, CD7, CD34, CD56, CD99 and terminal deoxynucleotidyl transferase (TdT)[8-10]. The second category is the abnormal immunophenotype of B-cell precursor, which is only found in B-ALL, instead of the abnormal expression pattern of antigens of normal B cells. The application of FCM in detecting the B-ALL MRD mainly depends on identifying the LAIPs that are different from the normal B-cell precursor and with the following characteristics: cross-lineage antigen expression, asynchronous antigen expression, enhanced or weakened expression of antigen[6,11]. Common immune markers for detection of B-ALL MRD include CD10, CD19, CD20, CD22, CD34, CD38, and CD45; CD58, CD81, CD73 and CD86 are also helpful in MRD detection by FCM[6,12-14]. Van Dongen et al[7] found that alterations may occur in the expres-

Table I. Characteristics of the common detection method for ALL-MRD.

Detecting the abnormal immunophenotype of leukemia using FCM	Detecting Ig/TCR gene rearrangement using RQ-PCR	Detecting the transcription of fusion genes using RQ-PCR	Detecting Ig/TCR gene rearrangement using NGS
Sensitivity			
3-4 colors: 10^{-3}-10^{-4}	10^{-4}-10^{-6}	10^{-4}-10^{-6}	10^{-6}
6-8 colors: 10^{-4}			
≥ 8 colors:10^{-4}-10^{-6}			
Suitability:			
B-ALL: > 90%	B-ALL: > 95%	B-ALL: 25-40%	ALL: > 95%
T-ALL: > 90%	T-ALL: > 95%	T-ALL: 10-15%	
Advantages:			
Rapid	High sensitivity	High sensitivity	Rapid
High suitability	High suitability	Simple and rapid	High sensitivity
MRD quantitative analysis in early stage	High specificity	Stable target gene	Not rely on specific primer
Access to other information of tumors	High degree of standardization	Suitable for leukemia of specific subtype (BCR-ABL,MLL-AF4, etc.)	Operation repeatable
	Stable DNA		Potential to recognition of oligocloning and evolutionary Analysis of genetic diversity and clonal heterogeneity
Disadvantages:			
Low sensitivity of 3 or 4 colors FCM	High cost	Limited suitability	Correct for disproportional PCR amplification of rearrangements
Need training and experience	Possessing abundant experience and knowledge	Difference in expression levels	
Immunophenotype transformation			
Limited standardization	Time-consuming	False positive results caused by PCR contamination	Visualization of data Analysis
			Limited experience in the field
	No analysis of MRD at an early stage	Poor stability	Higher cost
	False negative result caused by clonal evolution	Limited standardization	Lack of standardization

sion of surface antigen of leukemia cells during the treatment and recurrence, and, thus, suggested that the combination therapy of multiple antibodies should be carried out and the outcomes should be analyzed carefully to avoid the false negative results caused by the conversion of immunophenotypes[7]. A recent study shows that newly identified $CD34^{-dim}$ pre-B-I cells can be mistaken for residual BCP-ALL cells, potentially resulting in false-positive MRD outcomes, suggesting that it should be used as reference frame in MRD measurements by FCM[15]. The third category is the fusion protein expressed by fusion gene, such as BCR-ABL1, ETV6-RUNX1, TCF3-PBX1, and MLL/AF4[16,17]. At present, multi-parameter flow cytometry (MPFC) is one of the common methods for detecting the ALL-MRD. Burnusuzov et al[18] found that the standard 8-color FCM is applicable to the ALL-MRD detection in children for its capability to maximally reduce the negative impact of immune regulation therapy and to increase the sensitivity of analysis. However, multi-parameter FCM (not less than 8 colors), with the continuous update in the technique and method of FCM detection, will gradually replace the current FCM detection method. In addition, HLA-Flow is a FCM-based method using anti-HLA antibodies against mismatched HLA alleles combined with the antibodies against antigens expressed on leukemic cells. It is a sensitive, fast, and inexpensive method for the detection of MRD in patients with HLA-mismatched hematopoietic stem cell transplantation (HSCT)[19].

RQ-PCR

RQ-PCR is one of the most sensitive methods for detecting MRD, and its sensitivity can be as high as 10^{-6}. In MRD detection by PCR, sequence of Ig/TCR gene rearrangement or fusion gene is often used as the genetic marker for distinguishing the ALL cells from the normal cells.

1-Ig/TCR Gene Rearrangement Detection by RQ-PCR

The gene of the receptor of Ig/TCR antigen consists of the various discontinuous segments. V-(D)-J junctional regions produced by Ig and TCR gene rearrangements are characterized by the clonal specificity of leukemia, and the sequences of junctional region of different normal lymphocytes are different, but there is no gene rearrangement in the receptor of antigen of non-lymphocytes, through which these cells could be identified[20]. ALL is a kind of malignant clonal disease and the same or similar Ig/TCR gene rearrangement exists in ALL cells of each patient. Hence, the sequence of junctional region that has been identified in the diagnosis could be used for tracking the variations in MRD [21]. Van Dongen et al [7] reported that the incidence rates of IGH, TRB and TRG gene rearrangement in child patients with B-ALL were respectively 98%, 33% and 55%, while the rates in T-ALL were 23%, 92% and 95%. The stability of detection using IGH and IGK for child patients with B-ALL could be as high as 88% and 95%, and the stability of detection using TRB, TRG and TRD for child patients with T-ALL could be as high as 80%, 86% and 100%. Although this method is characterized by high sensitivity, specificity and stability and suitable for most of the patients, it also has some disadvantages, such as high cost, specific primer, excessively long time for screening, clonal evolution and oligoclone in Ig/TCR gene rearrangement, which will arise the false negative results. Thus, to attain a higher precision in detection of MRD, it is recommended that at least two kinds of Ig/TCR target molecules should be applied in the MRD detection of each patient [22].

2-Detection of Fusion Gene Using RQ-PCR

Fusion gene, a good index for detection of MRD, is a kind of specific molecular marker of leukemia that is generated by the chromosome translocation. Accurate detection of leukemia subtype that contains fusion genes can be carried out using RQ-PCR, but only applicable to the about 40% of ALL patients with specific and abnormal molecular biological features. There are 4 kinds of most common fusion genes in pediatric ALL, including TEL/AML1 fusion gene (also known as ETV6-RUNX1) that is generated by translocation of t (12;21), TCF3/PBX1 fusion gene, that is generated by translocation of t (1;19), BCR/ABL fusion gene, that is generated by translocation of t (9;22), and AF4/MLL fusion gene, that is generated by translocation of t (4;11) [16,23]. In B-ALL child patients, the incidence rates of the first two fusion genes are the highest, while the incidence rate of BCR/ABL fusion gene is the highest in the chronic myelogenous leukemia, but relatively low in the pediatric ALL. AF4/MLL fusion gene accounts for more than 2/3 in the infant leukemia. Ajuba et al[24] reported that there is a certain difference in comparison of detection rate of different fusion genes in different regions of the world, which might be caused by the genetic

diversity among different races . RQ-PCR, despite of its advantages in detection of fusion genes, such as rapid and simple operation, high specificity and sensitivity, also shows some disadvantages. For instance, RQ-PCR is only applicable to the specific subtype of leukemia; difference also exists in the expression of fusion genes in different individuals; PCR contamination can also arise the false positive result; PCR also has a poor stability; the ratio of PCR product to the quantity of leukemia cells is difficult to be established through RQ-PCR. Thus, accurate quantitative assay of MRD on cellular level cannot be achieved using RQ-PCR.

IR-SEQ

Next-generation sequencing (NGS), as well as the high-throughput sequencing (HTS), is emerging as a new flexible method for very sensitive sequencing analysis, and frequently used to detect the MRD in the diagnosis of leukemia clone through sequencing the rearranged genes of Ig and TCR with general primers [7,25]. With this technique, NGS can be monitored during the treatment; the sequencing of all rearranged genes in leukemia can be realized in given samples, the genes with clonal evolution can be detected in the follow-up samples, and the false negative results can also be reduced. The sensitivity of NGS can be as high as 10^{-6}, and specific primer of patient is not necessary, which, with shorter sequencing time, simple operation, high throughput and stability, and accessibility of standardization, overcomes the shortages of FCM and RQ-PCR in a certain degree. Pulsipher et al[26] compared the effects of high-throughput sequencing (HTS) and FCM respectively on the hematopoietic stem cell transplantation of patients with pediatric and adolescent ALL; the results showed that compared to the FCM, HTS is more accurate in predicting the recurrence and evaluating the prognosis before and after transplantation. Nevertheless, the cost of NGS is relatively high, which is a disadvantage of NGS[7, 26-28]. Currently, NGS, despite of its various advantages, has been scarcely applied in the clinical practices. However, at present, this method is only available in a few laboratories and is firm-dependent. Of note, NGS can also be applied to the detection of low levels of other chromosomal anomalies such as Ph- like alterations.

Clinical Application of MRD

A variety of studies have confirmed that consecutively monitoring the MRD during the treatment of patients with pediatric ALL is beneficial to the assessment of response to the therapy in an early stage, MRD-based risk grouping, and guiding the individualized treatment. The detection of MRD at the key time point via highly effective methods can also benefit the prognosis judgment and prediction of recurrence. In addition, the detection of MRD is of great clinical significance for the stem cell transplantation and treatment of recurrent ALL.

Assessing Early Response and Conducting MRD-Based Risk Stratification

In recent years, several studies have shown that MRD plays an important role in evaluating early therapeutic responses, and patients are divided into different risk groups and adjusted groups according to the level of MRD. Several investigations show that patients are divided into three groups: MRD level higher than 10^{-2} as the high-risk group; MRD level between 10^{-4} and 10^{-2} as the intermediate-risk group; MRD level lower than 10^{-4} as the low-risk group[29]. St. Jude Children's Research Hospital set the levels of MRD in bone marrow on the 15th and 42nd days in the treatment as the reference for MRD-based risk grouping. On the 42nd day, if the level of MRD of the patient with ALL in the standard-risk group is higher than 10^{-4}, the patient will be enrolled into the high-risk group[30]. Pui et al [31] performed the grouping according to the MRD levels on the 19th and 46th days of the remission induction: patients whose the level of MRD on the 19th day of remission induction are not less than 10^{-2} or on the 46th day are between 10^{-4} and 10^{-3} will be enrolled into the standard-risk group; patients whose the level of MRD in the end of remission induction are not less than 10^{-2} or after 7 weeks of treatment are not less than 10^{-3} will be enrolled into the high-risk group; the rest will be enrolled into the low-risk group. An International Randomized Trial (AIEOP-BFM ALL 2000) defined the standard-risk ALL as the absence of high-risk cytogenetics and undetectable MRD on days 33 and 78[32]. The Chinese Children Leukemia Group (CCLG) recommends that bone marrow (BM) was tested for MRD on day 33 and week 12, and SR patients with day 33 MRD $\geq$ 0.01% and < 1% were upstaged to IR, and patients with day 33 MRD $\geq$ 1% or week 12 MRD $\geq$ 0.1% were upstaged to HR[33,34]. Lately, Dana-Farber Cancer Institute (DFCI) has tested a new risk stratification system in children, patients who after achieving complete remission (CR) with high end-induction MRD were reclas-

sified as very high risk (VHR)[35]. Salina et al[36] showed that the MRD level in peripheral blood that was detected on the 8th day had the potential to predict the risk-based grouping for high-risk patients that were confirmed by the MRD level in bone marrow on the 15th day, and the high-risk patients with a rapid response can be isolated from those with a slow response in the patients with the MRD level of 10^{-2} on the 8th day, which could provide an opportunity for the intensified treatment in an early stage. Also, MRD was monitored after induction and consolidation Phase IB for measuring response[37]. In conclusion, although there remain some differences of MRD detection methods, detection time points and grouping standards among all research groups, an exact MRD cutoff of 10^{-4} has been taken as a critical value of low-risk group by many research groups, and the effect of MRD level on adjusting the intensity of treatment has also been highly appreciated. However, researches[38] have also shown that a single cut-off for MRD-based risk group does not reflect the response of the different genetic sub-types; the risk assessment program should combine genetics and MRD to accurately identify patients with the risk of relapse.

Evaluating Prognosis and Predicting Recurrence

At present, it is generally acknowledged that MRD is an independent prognostic factor for pediatric ALL, and a sensitive indicator of prediction of recurrence. The level of MRD lower than 10^{-4} was associated with better outcome[39]. A recent meta-analysis[40] confirmed that the lower the MRD, the better the prognosis . Also, the earlier MRD negativity is achieved, the lower the risk of relapse. In comparison with MRD positive patients at any level, patients who achieved MRD negativity at consolidation Phase IB had a low relapse risk, whereas those who attained MRD negativity at a later date showed higher cumulative incidence of relapse (CIR)[37]. Pui et al[41] conducted the MRD-based risk grouping according to the guidelines of National Cancer Institute (NCI), and found that the best prognosis could be attained in ALL patients with ETV6-RUNX1 fusion genes or hyperdiploid > 50, and recurrence risks are extremely low in these patients with a negative result of MRD detection on the 19th day. However, the high-risk B- or T-ALL patients suffer a poor prognosis even though their MRD detection on the 46th day is negative, and their accumulated recurrence risks are 12.7% and 15.5%, respectively. For B-ALL patients in standard risk, the overall prognosis is relatively moderate, but poor prognosis has also been observed if their MRD level are not less than 10^{-2} on the 19th day or MRD in any level is detected on the 46th day[41]. Similar conclusion has also been reported by Conter et al[42]. Some literatures also reported that the MRD level in the end of induction therapy (the 33rd day) is more significant for the prognosis judgment of B-ALL, while the MRD level in the end of consolidation therapy (the 78th day) will be more meaningful to the prognosis judgment of T-ALL[43,44]. Also, MRD detection by MFC and RQ-PCR detection of BCR-ABL at the early stage were important predictors of outcome in Ph^+-ALL, and these tests played complementary roles in predicting prognosis[45].

Application of MRD in the Stem Cell Transplantation

Quantitative assay of MRD levels before and after transplantation can not only effectively evaluate the prognosis and predict the recurrence, but also assist the selection of opportunity for transplantation in clinical practice. MRD negative before transplantation was associated with better outcome; persistence of any MRD level at pre-HSCT was associated with worse prognosis; patients with unchanged negative MRD both at pre-HSCT and at post-HSCT time point had the best EFS probability [46]. The MRD level after transplantation is closely associated with the recurrence[7]. Before transplantation, the MRD level is negatively correlated with the EFS, and positively correlated with the accumulated incidence rate of death caused by non-recurrence factors, but is not significantly correlated with the accumulated recurrence rate. After transplantation, MRD level at any time point is negatively correlated with the EFS, and positively correlated with the accumulated recurrence rate. The fitting analysis of multivariate Cox model at each time point showed that the MRD $\geq 10^{-4}$ is closely related to the poor EFS[47]. Umeda et al[48] retrospectively analyzed 38 cases of pediatric ALL patients who received the Allo-HSCT for the first time between 1998 and 2014 (33 CR cases and 5 non-CR cases); 33 CR cases were divided into the MRD negative group ($< 10^{-4}$) and MRD positive group ($\geq 10^{-4}$) based on the MRD level detected via FCM before transplantation. Results have shown that after transplantation, there are significant differences in comparisons of 3-year EFSs between the CR group and non-CR group as well as between the MRD negative group and MRD positive group;

two cases in the MRD positive group suffered the recurrence in 1 year after HSCT, and poor clinical prognosis was observed in patients of MRD positive group and non-CR group. Jabbour et al[49] showed that the EFS and OS of MRD negative patients who received the HSCT in S1 were significantly better than those of patients who received the HSCT in S2[49]. Pulsipher's et al[26] findings showed that better prognosis was acquired in the MRD negative patients who received the HTS detection before transplantation, and less-intensified treatment could be performed for these patients; on the 30th day after transplantation, the recurrence rate in HTS-MRD positive pediatric patients was significantly higher than that in the HST-MRD negative patients, and the multifactorial analysis showed that recurrence risk would be increased at any time after transplantation for MRD positive patients[26]. Also, Kotrova et al[50] showed that NGS has a better specificity in post-SCT ALL management and indicated that treatment interventions aimed at reverting impending relapse. Moreover, the MRD level after transplantation also has a great value for guiding the intervention treatment, guiding the infusion of lymphocytes for donor and the application of ciclosporin[51]. Besides, it has been showed[52] that in patients with minimal residual disease at pre-transplantation, the probability of OS after receipt of a transplant from a cord-blood donor was at least as favorable as that after receipt of a transplant from an HLA-matched unrelated donor and was significantly higher than the probability after receipt of a transplant from an HLA-mismatched unrelated donor. Furthermore, the probability of relapse was lower in the cord-blood group than in the other groups . In general, regardless of the pre- or post-transplantation, the lower the MRD level reaches, the earlier the intervention is implemented after transplantation, the better the prognosis will be.

Application of MRD in the Treatment for Recurrence of ALL

For patients with recurrence of ALL, MRD level is an important independent prognostic factor and can also be used to guide the treatment for ALL. Researchers in St. Jude Children's Research Hospital conducted the MRD detection for 35 patients with recurrent ALL after the second complete remission and found that the 2-year accumulated incidence rate of second recurrence in patients with MRD $\geq 10^{-4}$ was significantly higher than MRD $< 10^{-4}$. In patients who had experienced the first recurrence after treatment, about half of the patients with MRD $\geq 10^{-4}$ suffered the second time of recurrence, but no recurrence was observed in patients with MRD $< 10^{-4}$. In the recurrent patients who only received the chemotherapy, the recurrence rates of patients in two groups were 82% and 25%, respectively. Multi-parameter analysis showed that the occurrence time and MRD level in the first time of recurrence were two significant prognostic factors[53]. In another study, recurrence is significantly associated with MRD $\geq 10^{-6}$ and suggest that NGS for MRD detection can predict long-term relapse-free survival (RFS) in patients undergoing auto HCT for high-risk ALL[54]. For ALL that recurs after the second complete remission, MRD detection could also be used to guide the selection of treatment procedures after the second complete remission[55]. There are also some studies showing that better prognosis will be achieved if the MRD is negative in S1 of the patients with recurrent ALL regardless of the MRD level, and the 2-year overall survival rate is 65%, but the prognosis in patients in S2 is generally poor[49].

Guiding the Individualized Therapy

Patients with varying levels of MRD, different treatment procedures will have obviously distinct outcomes[56]. At present, clinical trials are still considering the adjustment of treatment regimen, that is, therapy reduction or allo-SCT based on MRD levels. Less-intensified treatment was carried out for the patients in standard risk group, and intensive treatment for patients in moderate risk group and high risk group. The results showed that 5-year EFS in the standard risk group was significantly higher than those in other two groups[33,57,58]. AIEOP-BFM ALL 2000 has shown that, while a better prognosis has been achieved for reduced chemotherapy intensity in the standard-risk group, the rate of recurrence has also increased[32]. ALL-2003 trial group in England also adopted the treatment procedures similar to AALL0232; the results showed that the 5-year EFS of patients with MRD level $\geq 10^{-4}$ who received the intensive treatment was 89.6%, superior to 82.8% of patients who received the standardized treatment[59]. Children's Oncology Group in US adopted the AALL0232 procedure for treatment of high-risk B-ALL patients and monitored the MRD levels in different stages; the results showed that 5-year EFS in pediatric patients with the MRD level $< 10^{-4}$ in the end of induction therapy could reach (87% ± 1%), and in patients with MRD level

between10^{-4} and 10^{-3} was (74% ± 4%); the EFS in patients whose MRD turned into negative from positive in the end of consolidation therapy was as high as (79% ± 5%), but the EFS in patients with MRD level ≥ 10^{-4} was only (39% ± 7%). Intensive treatment, although failed to improve the 5-year EFS or OS in patients with MRD level > 10^{-3}, altered the disease course of patients with positive MRD, providing an opportunity for adoption of further intervention[56]. Thus, dynamically monitoring the MRD level could directly reflect the load of leukemia cells in patients and assist the stipulation of treatment procedure. Based on the MRD levels, appropriate procedures could be applied to reduce the intensity of treatment for specific subgroups patients with a low level of MRD or negative MRD to alleviate the toxicity effect, and intensive treatment or HSTC is performed for patients with a high level of MRD to improve the prognosis[7,32]. In addition, the use of new agents such as monoclonal antibodies, small inhibitors, and chimeric antigen receptor T cells, is opening a new era of MRD-directed therapies, that will further increase survival rates[60].

Conclusions

Great progresses have been made in the detection method and clinical application value of MRD. On one hand, FCM and RQ-PCR are still the major detection techniques at present, and the combination of them, with their complementary advantages in technique, is applicable to more subjects and can increase the detection rate of MRD[61]. Nevertheless, the dominant position of these two classical methods is affected by NGS. NGS not only has a high sensitivity (10^{-6}), but also effectively overcomes the shortages in FCM and RQ-PCR. However, this method is also challenged by the difficulty in unifying the standardization of results in different experiment centers[62], which will be resolved with the wide application of NGS. On the other hand, MRD is not only the key and independent factors affecting the prognosis judgment, but also a sensitive indicator of prediction of recurrence; dynamically monitoring the MRD levels can not only evaluate the response to therapy in an early stage, direct the risk-based grouping, but also adjust the treatment procedure timely and guide the individualized treatment. Besides, MRD is also significant to the treatment of recurrent ALL and stem cell transplantation. With the development in detection technique, the new-generation, reliable and standardized MRD detection method will be widely applied in the clinical practices, guidance for treatment, improving the efficacy, extending the disease-free survival period of child patients and increasing their survival quality.

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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