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Clinical application of next-generation sequencing-based monitoring of minimal residual disease in childhood acute lymphoblastic leukemia

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Abstract

Background: Next-generation sequencing (NGS) is an emerging technology that can comprehensively assess the diversity of the immune system. We explored the feasibility of NGS in detecting minimal residual disease (MRD) in childhood acute lymphoblastic leukemia (ALL) based on immunoglobulin and T cell receptor. Methods: Bone marrow samples were collected pre- and post-treatment with pediatric ALL admitted to Shenzhen Children's Hospital from February 1st, 2020 to January 31st, 2021. We analyzed the MRD detected by NGS, multiparametric flow cytometry (MFC) and real-time quantitative PCR (RQ-PCR), and analyzed risk factors of positive NGS-MRD at the end of B-ALL induction chemotherapy. Results: A total of paired 236 bone marrow samples were collected from 64 children with ALL (58 B-ALL and 6 T-ALL). The decrease in the clonal rearrangement frequency of IGH, IGK, and IGL was generally consistent after treatment. Positive MRD was detected in 57.5% (77/134) of B-ALL and 80% (12/15) of T-ALL by NGS after chemotherapy, which was higher than those detected by MFC and RQ-PCR. In B-ALL patients, MRD results detected by NGS were consistent with MFC($r = 0.708$, $p < 0.001$)and RQ-PCR($r = 0.618$, $p < 0.001$). At the end of induction, NGS-MRD of 40.4% B-ALL was >0.01% and multivariate analysis indicated that [?]2 clonal rearrangement sequences before treatment were an independent factor of negative NGS-MRD. Conclusions: NGS is more sensitive than MFC and RQ-PCR for MRD measurement. B-ALL children with [?]2 clonal rearrangements detected by NGS before treatment are difficult to switch to negative MRD after chemotherapy.

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Acute lymphoblastic leukemia, children, minimal residual disease, next-generation sequencing

6. Abbreviations:

Abbreviations	Full spelling
ALL	acute lymphoblastic leukemia
BM	bone marrow
CDR	complementarity determining region
CNSL	central nervous system leukemia
COG	Children's Oncology Group
FDA	Food and Drug Administration
Hb	hemoglobin
IG	immunoglobulin
MFC	multiparametric flow cytometry
MRD	minimal residual disease
NEG	negative
NGS	next-generation sequencing
PB	peripheral blood
PLT	platelet
RQ-PCR	real-time quantitative PCR
TCR	T cell receptor

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Methods: Bone marrow samples were collected pre- and post-treatment with pediatric ALL admitted to Shenzhen Children's Hospital from February 1st, 2020 to January 31st, 2021. We analyzed the MRD detected by NGS, multiparametric flow cytometry (MFC) and real-time quantitative PCR (RQ-PCR), and analyzed risk factors of positive NGS-MRD at the end of B-ALL induction chemotherapy.

Results: A total of paired 236 bone marrow samples were collected from 64 children with ALL (58 B-ALL and 6 T-ALL). The decrease in the clonal rearrangement frequency of IGH, IGK, and IGL was generally consistent after treatment. Positive MRD was detected in 57.5% (77/134) of B-ALL and 80% (12/15) of T-ALL by NGS after chemotherapy, which was higher than those detected by MFC and RQ-PCR. In B-ALL patients, MRD results detected by NGS were consistent with MFC($r = 0.708$, $p < 0.001$)and RQ-PCR($r = 0.618$, $p < 0.001$). At the end of induction, NGS-MRD of 40.4% B-ALL was >0.01% and multivariate

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Conclusions: NGS is more sensitive than MFC and RQ-PCR for MRD measurement. B-ALL children with [?]2 clonal rearrangements detected by NGS before treatment are difficult to switch to negative MRD after chemotherapy.

Keywords: Acute lymphoblastic leukemia, children, minimal residual disease, next-generation sequencing

1 — INTRODUCTION

Minimal residual disease (MRD) is a recognized index for monitoring the effect of treatment in childhood acute lymphoblastic leukemia (ALL), and it is the most reliable and independent prognostic factor of ALL[1-3]. Multi-parameter flow cytometry (MFC) and real-time quantitative PCR (RQ-PCR) are the most used methods to detect ALL MRD, but MFC is operator- and laboratory-dependent and leukemic antigen expressions altered after chemotherapy may invalidate MRD marker[4]. The clonal rearrangement of the variable region of IG/TCR or fusion genes is used as a marker for MRD detection using RQ-PCR. The former requires the design of patient-specific primers for clonal rearrangement based on the variable region of IG/TCR, which is expensive and labor-intensive, while the latter has limited application because only 1/3 of patients have leukemia-specific translocations[5].

The sensitivity and reliability of IG/TCR detected using NGS for ALL MRD has been demonstrated[6]. The sensitivity of NGS, which can reach 10^{-6}-10^{-7}, depends on the number of DNA in the sample, and NGS is suitable for roughly 90% of ALL. There is no need to design patient-specific primers, and the cloned evolutionary can be detected in the follow-up samples to reduce false negative results[7-11]. It is worth mentioning that previous studies on MRD detection based on NGS have found that NGS is more accurate in risk stratification and prediction of recurrence of ALL than traditional techniques in both chemotherapy and transplant children ALL[12, 13]. At the same time, how the clonal repertoire of ALL changes throughout the progression of the disease can be monitored[14, 15]. However, the changes of different types of clonal rearrangements after the treatment and the significance of NGS in the clinical application still need to be further explored. In this study, we analyzed the changes of IGH, IGK and IGL clonal rearrangement frequency after treatment, compared the results of MRD detection by NGS, MFC and RQ-PCR, respectively, and explored the risk factors of NGS-MRD at the end of induction in children B-ALL.

2 — MATERIALS AND METHODS

2.1 — Patients and samples

We analyzed 64 pediatric ALL (B-ALL, N=58; T-ALL, N=6) enrolled in the CCCG-ALL-2015 protocol (N=40) and CCCG-ALL-2020 protocol (N=24). Paired 236 bone marrow samples were collected before treatment, on day 19, at the end of induction and end of consolidation chemotherapy, and then MFC and NGS were used to find MRD markers and monitor MRD. Chromosomal translocations/gene fusions were detected in 18 ALL cases before treatment, which were detected fusion transcript using RQ-PCR at same time points after treatment. All samples were sent to Kinstar Global for testing. The study was approved by the ethics committee, and written informed consent was obtained from the parent or guardian of each child.

2.2 — MFC

MRD of bone marrow specimens by MFC used 8-color antibody combinations. A target of 1000000 events was analyzed per reagent combination, and MRD reported as a percentage of cells with abnormal surface protein expression with a sensitivity of 10^{-4}. Traceable leukemia-associated immunophenotyping was not detected in 5 out of 64 ALL, and simplified MFC was used to track MRD of the 5 ALL after treatment.

2.3 — RQ-PCR

Fusion genes with clear clinical significance at initial diagnosis were used as markers for the MRD examined.

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The cDNA was reverse transcribed with mRNA as the template and then PCR with cDNA as the template to detect the fusion genes. RQ-PCR was monitored for MRD with a sensitivity of 10^{-4} by adding primers and specific fluorescence probes while PCR amplification to quantify the fusion genes.

2.4 — NGS

The gDNA of single nucleated cells was extracted from bone marrow samples, and then multiple primers were designed to amplify CD3 region of IGH-VDJ, IGH-DJ, IGK and IGL of B-ALL or TRG and TRB of T-ALL. Add the identification required for sequencing for each sample, and then the amplification products were detected by NGS (Illumina technology sequencing platform) to obtain many reads. Sequencing results were analyzed to obtain the same fragment to form a clonotype. The following three criteria can be defined as the dominant index sequence: 1. The frequency of the clonal sequence is higher than 3%. 2. The ratio of the total nuclear cell content is higher than 0.2%. 3. the clone sequence presents a discontinuous distribution. Dominant index sequences were used as a marker for subsequent MRD evaluation with a sensitivity of at least 10^{-6}. Wuhan Kingstar Medical Inspection Co., Ltd. provided technology support for NGS MRD detection.

2.5 — Statistical method

Repeated measures of variance were used to compare the changes of the frequency of IGH, IGK, and IGL clonal rearrangements after treatment. The NGS-MRD was compared with the tumor burden measured by MFC and PQ-PCR, respectively, using the Pearson correlation. Categorical variables were analyzed with Chi-Square test or Fisher's exact test, and continuity variables meeting the normal distribution were analyzed by the t-test, while those not with normal distribution were analyzed by Mann-Whitney U test. Finally, logistics regression was used to analyze the risk factors for B-ALL NGS-MRD failure to turn negative at the end of induction remission chemotherapy.

3 — RESULTS

3.1 — Clonal sequence assessment

In the pretreatment samples of 64 patients (58 B-ALL, 6 T-ALL), 87.9% (51/58) B-ALL had at least one traceable clonal index sequence, with IGH clone rearrangement sequences being the most common, accounting for 86.2% (50/58). The detection rates of traceable IGK and IGL clone rearrangement sequences were 27.6% (16/58) and 12.1% (7/58) in B-ALL samples, respectively (Table1). Clone rearrangement was detected in all T-ALL patients. TRG clone rearrangements was detected in 66.7% (4 Universe 6) of the samples, and TRB was detected in half of the samples (Table1).

A total of 128 clone rearrangements (median 2, range 0-6 clones/patient) were detected in 58 patients with B-ALL, including 96 IGH, 24 IGK and 8 IGL. The decrease of the frequency of IGH, IGK and IGL clone rearrangement sequences after treatment were roughly parallel, and there was no significant difference in MRD values of various clonal sequences ($F = 0.307$, $p = 0.736$) (Figure1).

MRD of B-ALL

A total of 150 post-treatment specimens from 58 B-ALL were analyzed by NGS. 16 follow-up samples of 7 cases were excluded because clonal rearrangement sequences were not identified in their diagnostic samples. The tumor load of the remaining 134 bone marrow samples from 51 remaining patients (44 at D19, 47 at the end of induction and 43 at the end of consolidation chemotherapy) were analyzed. More positive MRD could be detected using NGS than conventional techniques at each stage (Figure 2).

To assess the diagnostic accuracy of NGS, we compared NGS and MFC**.** In total of 134 post-treatment samples were analyzed by both NGS and MFC, and there was concordance between the two methods for tumor burden levels ($r = 0.708$, $p < 0.001$). In qualitative analysis, the positive rate of NGS-MRD (77/134, 57.5%) was higher than that of MFC -MRD (36/134,26.9%) at the same time points. Positive and negative coincidence rates for NGS and MFC were 97.2% and 57.1%, respectively. Results were inconsistent in 43 of 134 follow-up samples, including 42 tested positive by NGS but negative by MFC—the median NGS-MRD value of these 42 samples is 2.77×10^{-4}(3.61×10^{-2}-2.06×10^{-7}). The remaining inconsistent case was MFC

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+/NGS-, whose MFC-MRD value was 5.00×10^{-4}, while the TEL-AML of this patient was tested negative by RQ-PCR at the same time. When setting the cutoff value of MRD to 0.01%, NGS could still detect more positive MRD than MFC (44%, 26.9%). (Figure3 A)

ALL associated fusion genes were detected in 18 of 51 B-ALL by RQ-PCR. 48 post-treatment samples of 18 B-ALL were detected by both NGS and RQ-PCR. Quantitative analysis of the results collected using the two techniques were in concordance to a certain extent (r=0.618, $p< 0.001$). Quantitative analysis of the results showed that the coincidence rates of positive and negative were 100% and 59.0%, respectively. More positive samples could be detected by NGS than by RQ-PCR (52.1%, 18.8%). None of the positive samples tested by MFC is negative by NGS. However, there were 16 cases with NGS+/PCR-, and the median NGS-MRD value of them was $5.00 \times 10^{-4}(3.61\times10^{-2}$ -$2.69\times10^{-6})$. The tumor burdens of 6 cases among the 16 cases were more than 0.01%. (Figure3 B)

3.3 — MRD of T-ALL

A total of 28 samples (6 pre-treatment and 22 post-treatment) from 6 children with T-ALL were sequenced by NGS, and clonal rearrangements were detected before treatment in all pre-treatment samples (TRB in 3/6 cases and TRG in 4/6 cases). Among 6 patients, no traceable leukemia specific antigen was found by MFC before treatment in one case. On day 19 after treatment**,** the tumor load was detected by a simplified flow cytometry as MRD < 0.01%, while the result of NGS was 1.41%. The follow-up 6 bone marrow samples of this case were only detected by NGS for the follow-up MRD, but not by MFC at the same time. So far, the NGS-MRD of the patient is still positive.

In the remaining 15 post-treatment samples, the positive rates of MRD detected by NGS and MFC were 80% (12/15) and 46.7% (7/15), respectively. No sample was tested positive by MFC-MRD but negative by NGS-MRD. However, 5 samples were tested positive by NGS-MRD but negative by MFC-MRD, of which 4 samples had NGS-MRD values of greater than 0.01%, and 1 sample had NGS-MRD value of $4.05\text{x}10^{-6}$.

3.4 — Risk factors affecting B-ALL MRD turning negative

Previous studies had confirmed that within the same threshold of MFC (0.01%), more patients with positive MRD would be identified by NGS than MFC at the end of induction, and the prognosis of these patients was worse than that of patient who were tested negative by both NGS-MRD and MFC-MRD[12]. This suggests that NGS may better stratify the risk of B-ALL. We set the threshold of tumor load detected by NGS to 0.01% (It means that MRD < 0.01 was considered negative) at the end of the induction in B-ALL. The clinical characteristics and laboratory indexes before treatment were analyzed to explore the risk factors affecting the negative conversion of MRD.

Among the 58 cases of B-ALL, no trackable clonal index sequence pretreatment was found in 6 cases, and 5 cases were excluded because the bone marrow of them was insufficient. The remaining 47 children with B-ALL were divided into two groups based on whether NGS-MRD turned negative at the end of the induction, of which 19 were MRD positive and 28 were MRD negative. The results of univariate analysis showed that the treatment regimen, the tumor load in bone marrow, platelet count and the number of clone index sequences at diagnosis were related to the conversion of MRD to be negative in B-ALL patients at the end of induction chemotherapy. The results of multivariate analysis showed that the NGS-MRD of patients with 2 or more clonal rearrangement sequences at diagnosis was more difficult to turn negative. (Table2, Table3.). Two or more clonal rearrangement sequences were detected in 76.6% (36/47) of patients, with 50% (18/36) turning negative for NGS-MRD, while the NGS-MRD of 90% (10/11) of patients with only one clonal rearrangement sequence turned negative.

4 — DISCUSSTION

NGS overcomes the limitations of conventional techniques and has great potential in detecting ALL MRD. However, NGS technology is challenged by accurate identification of leukemia-specific IG/TCR clones. The average frequency of IGH rearrangements in healthy samples was 0.08%+-0.04% of total nucleated cells. Wu *et al.* [16]defined the sequence frequency of > 10% as the threshold for leukemia-specific sequences and

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detected the leukemia-specific sequence in 93.9% (92/98) B-ALL from Pediatric Oncology Group (COG), while more studies[12, 17-19] set the threshold of 5%, in which IGH rearrangements in around 90% of the B-ALL were seen. A study from COG[12] sequenced the IgH and TRG CDR3 regions of the B-ALL using the ImmunoSEQ platform (Adaptive BioTechnologies, Seattle, WA) and detected dominant index sequences in 95.4% (579/607) of B-ALL pre-treatment samples, where 89% had IGH rearrangements, and only TRG, which was used as cross-lineage rearrangements to monitor MRD, was detected in 6.4% of the samples. ClonoSEQ is the first NGS platform approved by the Food and Drug Administration (FDA) for MRD evaluation in B-ALL and multiple myeloma (MM) patients, which defines the traceable dominant index sequence as: it is at least 3% as a percentage of all sequences in the locus, it must also have a frequency of at least 0.2% of all nucleated cells in the sample and must have sufficient abundance and differentiation from a polyclonal background[20]. We utilized this criterion and sequenced based on the rearrangement of the CD3 region of IgH (VDJ), IgH (DJ), IgK, and IgL without TRG to detect the MRD of B-ALL. Our study suggested that at least one clonal rearrangement was detected in 87.9% of the samples before treatment, and similar to previous studies, IGH was the most common clonal rearrangement. In addition, we found that the downward trends of clonal rearrangements IgH, IgK and IgL were consistent and there was no significant difference in their representative tumor burden.

NGS has high sensitivity and accuracy in monitoring the treatment response of ALL [18]. The positive rate of NGS-MRD detected after treatment was about twice that of MFC-MRD in our study, and inconsistent results included high levels ([?]0.01%) and low levels (<0.01%) of tumor load. About one-quarter of the positive samples determined by NGS-MRD in this study were low-level MRD (MRD <0.01%). Long-term follow-up studies found that low levels of MRD after chemotherapy were significantly associated with poor prognosis of ALL after chemotherapy [21]. Due to the detectable low levels of MRD by highly sensitive NGS, the positive MRD detected by NGS in the late stage of treatment can more accurately predict poor prognosis than in the early stage of treatment[17, 22]. The first clinical study applying NGS to MRD monitoring after complete B-ALL remission by Cheng *et al.* [23] confirmed that the timing of NGS-MRD transposition in relapsed patients (on average 25.6 weeks before clinical relapse) was significantly earlier than MFC-MRD and accurately predicted relapse, which expanded the treatment window for relapsed patients.

To determine the optimal cutoff point for risk stratification using NGS-MRD, Wood B *et al* .[12] examined bone marrow samples from 579 children at the end of B-ALL induction from COG to calculate the Cox proportional hazard ratios at different NGS-MRD cutoff threshold, and the MRD cutoff point providing the maximum risk ratio was 0.01%. The study also found that at the end of the induction, NGS had identified approximately one-third more MRD-positive patients than MFC within this threshold. Moreover, the long-term EFS in these patients were significantly lower than that of patients with negative MRD by both MFC and NGS (P =0.036). Previous large sample studies of COG[24] and St. Jude Children's Research Hospital[25] suggested that the most appropriate time point for childhood ALL using MRD for risk assessment and guiding stratified treatment was the end of induction. In our study, at the end of the induction, 8 out of the 19 samples with NGS-MRD values greater than 0.01% had a negative MFC-MRD. The prognostic situation in this group also requires further follow-up to confirm the prognostic value of NGS-MRD. As there was no recurrence or death, and the follow-up time was short (the median follow-up time of B-ALL in the study was 15 months), we did not perform a survival analysis. Further long-term follow-up was needed to confirm the reliability of NGS-MRD in the group. We used 0.01% as the cutoff value of NGS-MRD at the end of the induction and multivariate analyzed the factors affecting NGS-MRD turning negative. The result showed that MRD in children B-ALL with two or more clonal rearrangement sequences were more difficult to turn negative.

The RQ-PCR based on the rearrangement of the Ig/TCR gene has been thoroughly standardized through international cooperation[26], and RQ-PCR MRD is also considered as a reliable indicator of relapse prediction post-allotransplant and is the basis for prophylactic therapy[27]. Many literatures had reported that the correlation between the MRD results determined by NGS and RQ-PCR was satisfactory [28, 29]. But the literature reported[30] that after the low level and unquantifiable positive MRD results determined by RQ-PCR testing, as a gray area with high false positive probability, were retested by NGS with greater sequencing

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depth, the majority of cases identified as negative by NGS did not relapse, while most low positive MRD samples from relapsed patients were confirmed positive for MRD by NGS, indicating that NGS has better specificity for MRD detection after transplantation. In this study, MRD results collected from RQ-PCR based on fusion gene was compared with NGS-MRD and showed good correlation.

However, the standardization of NGS is still ongoing[31]. EuroClonality-NGS is working to standardize NGS assays for IgH/TCR MRD identification[32, 33]. A multicenter study[34], done by EuroClonality-NGS, validated the results of the IG clonal analysis of NGS between different laboratories, and the results from NGS were also compared with conventional EuroClonality/ BIOMED-2 GeneScan clonality analysis. The concordance was 99% and 98%, respectively.

In summary, as a relatively novel method to identify molecular markers in most of ALL patients, NGS is an effective and highly promising tool for MRD detection in ALL. Based on its technical advantages, the NGS platform may soon become a reliable method to complement existing techniques for MRD evaluation of lymphoid tumors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

[1] Modvig S, Madsen HO, Siitonen SM, et al. Minimal residual disease quantification by flow cytometry provides reliable risk stratification in T-cell acute lymphoblastic leukemia. Leukemia. 2019. 33(6): 1324-1336.

[2] Donald A. Berry P, Shouhao Zhou P, Howard Higley P, et al. Association of Minimal Residual Disease With Clinical Outcome in Pediatric and Adult Acute Lymphoblastic Leukemia AMeta-analysis. 2017. 3(7): e170580.

[3] Ceppi F, Rizzati F, Colombini A, Conter V, Cazzaniga G. Utilizing the prognostic impact of minimal residual disease in treatment decisions for pediatric acute lymphoblastic leukemia. Expert Rev Hematol. 2021. 14(9): 795-807.

[4] Roshal M, Fromm JR, Winter S, Dunsmore K, Wood BL. Immaturity associated antigens are lost during induction for T cell lymphoblastic leukemia: implications for minimal residual disease detection. Cytometry B Clin Cytom. 2010. 78(3): 139-146.

[5] Lazic J, Tosic N, Dokmanovic L, et al. Clinical features of the most common fusion genes in childhood acute lymphoblastic leukemia. Med Oncol. 2010. 27(2): 449-453.

[6] Wu D, Sherwood A, Fromm JR, et al. High-throughput sequencing detects minimal residual disease in acute T lymphoblastic leukemia. Sci Transl Med. 2012. 4(134): 134ra63.

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[7] Kruse A, Abdel-Azim N, Kim HN, et al. Minimal Residual Disease Detection in Acute Lymphoblastic Leukemia. Int J Mol Sci. 2020. 21(3): 1054.

[8] Kotrova M, Novakova M, Oberbeck S, et al. Next-generation amplicon TRB locus sequencing can overcome limitations of flow-cytometric Vβ expression analysis and confirms clonality in all T-cell prolymphocytic leukemia cases. Cytometry A. 2018. 93(11): 1118-1124.

[9] Sala Torra O, Othus M, Williamson DW, et al. Next-Generation Sequencing in Adult B Cell Acute Lymphoblastic Leukemia Patients. Biol Blood Marrow Transplant. 2017. 23(4): 691-696.

[10] Ladetto M, Brüggemann M, Monitillo L, et al. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. Leukemia. 2014. 28(6): 1299-1307.

[11] Qin X, Zhang MY, Liu WJ. Application of minimal residual disease monitoring in pediatric patients with acute lymphoblastic leukemia. Eur Rev Med Pharmacol Sci. 2018. 22(20): 6885-6895.

[12] Wood B, Wu D, Crossley B, et al. Measurable residual disease detection by high-throughput sequencing improves risk stratification for pediatric B-ALL. Blood. 2018. 131(12): 1350-1359.

[13] Pulsipher MA, Carlson C, Langholz B, et al. IgH-V(D)J NGS-MRD measurement pre- and early post-allotransplant defines very low- and very high-risk ALL patients. Blood. 2015. 125(22): 3501-3508.

[14] Contreras Yametti GP, Ostrow TH, Jasinski S, Raetz EA, Carroll WL, Evensen NA. Minimal Residual Disease in Acute Lymphoblastic Leukemia: Current Practice and Future Directions. Cancers (Basel). 2021. 13(8): 1847.

[15] Coccaro N, Anelli L, Zagaria A, Specchia G, Albano F. Next-Generation Sequencing in Acute Lymphoblastic Leukemia. Int J Mol Sci. 2019. 20(12):2929.

[16] Wu D, Emerson RO, Sherwood A, et al. Detection of minimal residual disease in B lymphoblastic leukemia by high-throughput sequencing of IGH. Clin Cancer Res. 2014. 20(17): 4540-4548.

[17] Sekiya Y, Xu Y, Muramatsu H, et al. Clinical utility of next-generation sequencing-based minimal residual disease in paediatric B-cell acute lymphoblastic leukaemia. Br J Haematol. 2017. 176(2): 248-257.

[18] Faham M, Zheng J, Moorhead M, et al. Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood. 2012. 120(26): 5173-5180.

[19] Pui CH, Pei D, Coustan-Smith E, et al. Clinical utility of sequential minimal residual disease measurements in the context of risk-based therapy in childhood acute lymphoblastic leukaemia: a prospective study. Lancet Oncol. 2015. 16(4): 465-474.

[20] Monter A, Nomdedéu JF. ClonoSEQ assay for the detection of lymphoid malignancies. Expert Rev Mol Diagn. 2019. 19(7): 571-578.

[21] Stow P, Key L, Chen X, et al. Clinical significance of low levels of minimal residual disease at the end of remission induction therapy in childhood acute lymphoblastic leukemia. Blood. 2010. 115(23): 4657-4663.

[22] Logan AC, Vashi N, Faham M, et al. Immunoglobulin and T cell receptor gene high-throughput sequencing quantifies minimal residual disease in acute lymphoblastic leukemia and predicts post-transplantation relapse and survival. Biol Blood Marrow Transplant. 2014. 20(9): 1307-1313.

[23] Cheng S, Inghirami G, Cheng S, Tam W. Simple deep sequencing-based post-remission MRD surveillance predicts clinical relapse in B-ALL. J Hematol Oncol. 2018. 11(1): 105.

[24] Borowitz MJ, Devidas M, Hunger SP, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood. 2008. 111(12): 5477-5485.

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[25] Pui CH, Pei D, Raimondi SC, et al. Clinical impact of minimal residual disease in children with different subtypes of acute lymphoblastic leukemia treated with Response-Adapted therapy. Leukemia. 2017. 31(2): 333-339.

[26] Langerak AW, Groenen PJ, Brüggemann M, et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. Leukemia. 2012. 26(10): 2159-2171.

[27] Bader P, Kreyenberg H, von Stackelberg A, et al. Monitoring of minimal residual disease after allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia allows for the identification of impending relapse: results of the ALL-BFM-SCT 2003 trial. J Clin Oncol. 2015. 33(11): 1275-1284.

[28] Kotrova M, Muzikova K, Mejstrikova E, et al. The predictive strength of next-generation sequencing MRD detection for relapse compared with current methods in childhood ALL. Blood. 2015. 126(8): 1045-1057.

[29] Ferrero S, Ladetto M, Drandi D, et al. Long-term results of the GIMEMA VEL-03-096 trial in MM patients receiving VTD consolidation after ASCT: MRD kinetics' impact on survival. Leukemia. 2015. 29(3): 689-695.

[30] Kotrova M, van der Velden V H J, van Dongen J J M, et al. Next-generation sequencing indicates false-positive MRD results and better predicts prognosis after SCT in patients with childhood ALL. Bone Marrow Transplant. 2017. 52(7): 962–968.

[31] van Dongen JJ, van der Velden VH, Brüggemann M, Orfao A. Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. Blood. 2015. 125(26): 3996-4009.

[32] Brüggemann M, Kotrová M, Knecht H, et al. Standardized next-generation sequencing of immunoglobulin and T-cell receptor gene recombinations for MRD marker identification in acute lymphoblastic leukaemia; a EuroClonality-NGS validation study. Leukemia. 2019. 33(9): 2241-2253.

[33] Knecht H, Reigl T, Kotrová M, et al. Quality control and quantification in IG/TR next-generation sequencing marker identification: protocols and bioinformatic functionalities by EuroClonality-NGS. Leukemia. 2019. 33(9): 2254-2265.

[34] van den Brand M, Rijntjes J, Möbs M, et al. Next-Generation Sequencing-Based Clonality Assessment of Ig Gene Rearrangements: A Multicenter Validation Study by EuroClonality-NGS. J Mol Diagn. 2021. 23(9): 1105-1115.

TABLE 1 Clonal rearrangements detected in the pretreatment sample

	Clone rearrangement	Number of cases
B-ALL	Total	58
	No Clone rearrangement	7 (12.1%)
	IGH	32 (55.2%)
	IGH IGK	11 (19%)
	IGH IGL	3 (5.2%)
	IGH IGL IGK	4 (6.9%)
	IGK	1 (1.7)
T-ALL	Total	6
	TRB	2 (33.3%)
	TRG	3 (50%)
	TRG TRB	1 (16.7%)

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TABLE 2 Univariate analysis of the risk factors for B-ALL NGS-MRD turning negative at the end of induction remission chemotherapy

Parameters	Total (N=47)	MRD- (N=28)	MRD+ (N=19)	P-Value
Treatment protocol (CCCG-ALL2020)	27.7% (13/47)	39.3% (11/28)	10.5% (2/19)	0.031
Gender(male)	61.7% (29/47)	53.6% (15/28)	73.7% (14/19)	0.164
Age(years)	4.00 (0.25-11.75)	4.08(0.25-11.75)	4.00(0.50-11.25)	0.838
WBC(×10ˆ9/L)	8.79 (0.21-222.80)	7.47(0.88-109.5)	15.02(0.21-222.8)	0.461
Hb(g/L)	71 (25-125)	77.5(34-124)	57(25-135)	0.085
PLT(×10ˆ9/L)	42 (1-387)	55(1-387)	22(6-246)	0.015
Immature leukemia cells in PB (%)	40 (0-94)	18(1-94)	60(0-92)	0.07
Immature leukemia cells in BM(%)	92 (27-99)	89.25(49-99)	94.5(27-99)	0.045
Number of clonal rearrangement sequences >1	76.6% (36/47)	64.3% (18/28)	94.7% (18/19)	0.032
CNSL				0.642
CNS1	35	20	15	
CNS2	10	6	4	
CNS3	2	2	0	
Number of chromosomes				0.177
>50	3	2	1	
<50	29	20	9	
No split phase	15	6	9	
Fusion genes				0.783
BCR-ABL	1	1	0	
TEL-AML	7	3	4	
MLL	4	2	2	
TCF3-PDX1	4	3	1	
Negative	30	19	11	

Abbreviations: WBC=white blood cell; PLT= platelet; PB= peripheral blood; BM= bone marrow; CNSL= central nervous system leukemia.

TABLE 3 Multivariate analysis of the risk factors for B-ALL NGS-MRD turning negative at the end of induction remission chemotherapy at the end of induction remission chemotherapy.

Parameter	B	Wald	p	Exp(B)	95%CI
Number of clonal rearrangement sequences >1	2.367	4.484	0.034	10.664	1.193-95.354

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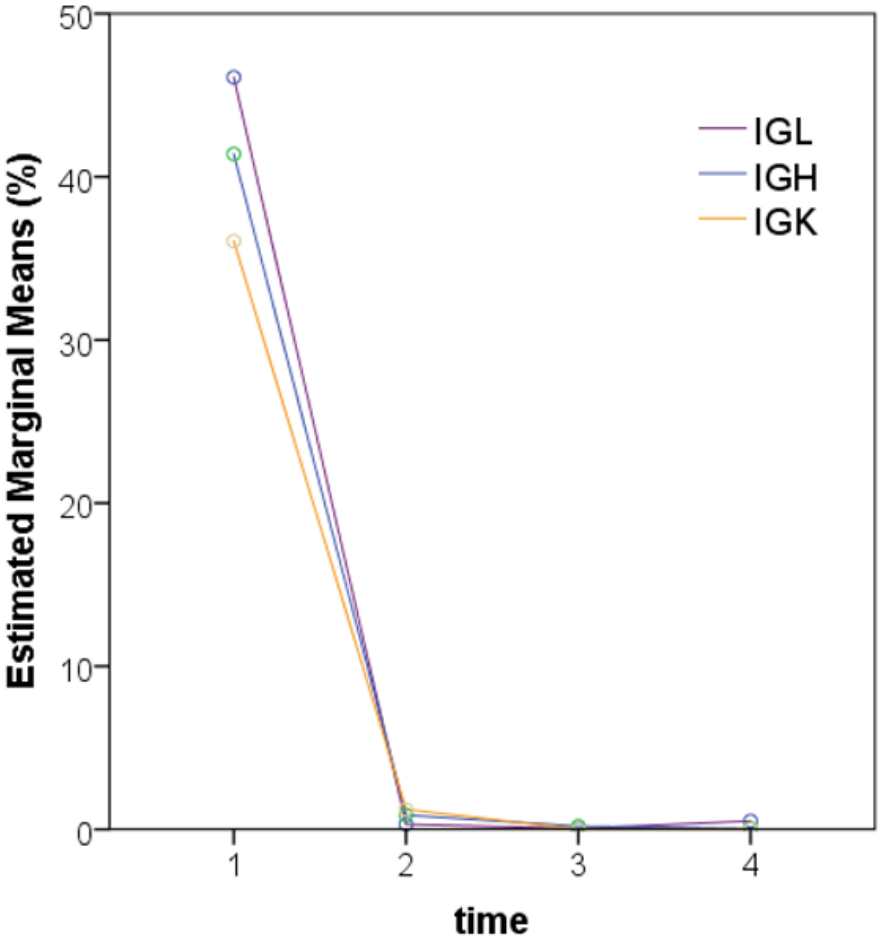

FIGURE 1 The changes of the frequency of IGH, IGK and IGL clonal rearrangements after treatment. Abscissa: 1=before treatment; 2=on day 19; 3=at the end of induction; 4=at the end of consolidation chemotherapy. The decrease of the frequency of IGH, IGK and IGL clone rearrangement sequences after treatment were roughly parallel, and there was no significant difference in MRD values of various clonal sequences (F=0.307, p =0.736).

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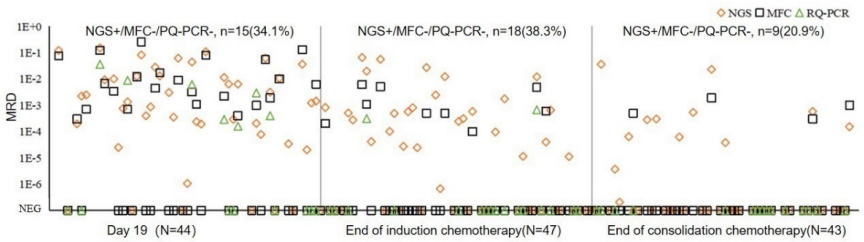

FIGURE 2 MRD values of B-ALL at different periods after chemotherapy. on day 19, at the end of induction chemotherapy and end of consolidation chemotherapy, NGS detected MRD positive specimens while MFC or RQ-PCR did not detect residual tumor cells were 15, 18 and 9 specimens respectively.

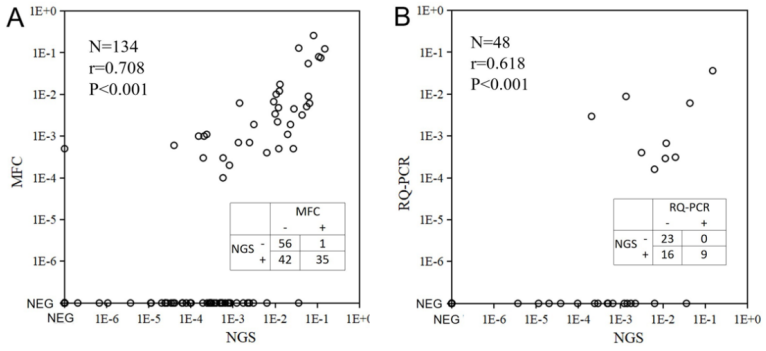

FIGURE 3 Correlation analysis of NGS with RQ-PCR and MFC results. (A): Correlation of MRD results detection by NGS and MFC; (B): Correlation of MRD results detection by NGS and RQ-PCR.

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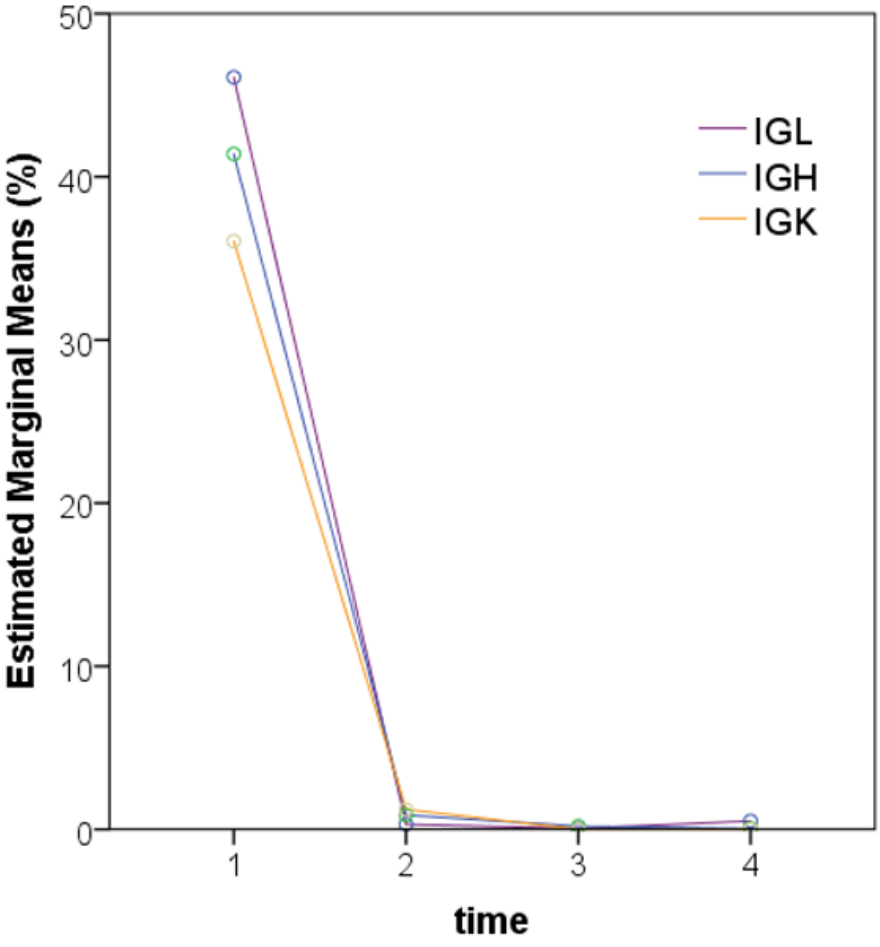

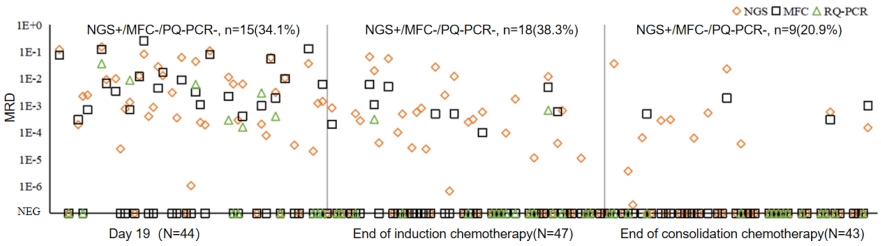

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