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A prospective evaluation of minimal residual disease as risk stratification for CCLG-ALL-2008 treatment protocol in pediatric B precursor acute lymphoblastic leukemia

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Abstract. – OBJECTIVE: The aim of this prospective study was to evaluate the cut-off value of minimal residual disease (MRD) in predicting the efficacy of CCLG-ALL-2008 or CCLG-2008 treatment protocol on pediatric B-precursor ALL (BP-ALL).

PATIENTS AND METHODS: Three hundred and seventy-nine Chinese pediatric BP-ALL were enrolled in this study between Dec 2008 and Sep 2013 in two stratified cohorts. One hundred and fifty-three patients enrolled between Dec 2008 and Oct 2010 as the first cohort, and 196 patients enrolled from Nov 2010 to Sep 2013 as the second cohort. Clinical and biological characteristics and 5 years EFS, RFS, and OS were analyzed.

RESULTS: Patients with E2A-PBX1 showed a favorable treatment response with a lower minimal residual disease (MRD) level (< 10^{-4}) at the time point 1 (TP1, $p = 0.039$) and the highest proportion of the 5-year EFS, RFS, and OS. A high level of MRD was associated with high WBC counts, increased age, BCR-ABL1 fusion gene, MLL rearrangements and adverse karyotypes. In comparison with the first cohort, the second cohort with the MRD assay incorporated prospectively, the standard risk (SR) and the intermediate risk (IR) patients showed a better RFS, EFS, and OS while the high-risk (HR) patients displayed worse RFS, EFS, and OS than those of the first cohort, respectively. Patients with MRD level at either 10^{-4} or 10^{-3} showed a similar OS at TP1 or TP2, and patients with MRD level above 10^{-2} had the worst OS.

CONCLUSIONS: This study indicated that the levels of MRD to be an adequate guide in risk-adapted treatment under the CCLG-ALL-2008 protocol and can be adapted to the future development of advanced clinical protocols.

Key Words:

Acute lymphoblastic leukemia (ALL), Minimal residual disease (MRD), Relapse-free survival (RFS), Event-free survival (EFS), Overall survival (OS).

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy diagnosed in children (aged 1-18 years), accounting for 75% to 80% of cases of acute leukemia among this age group[1]. Dramatic improvements in the cure rates and survival outcomes for pediatric ALL have been made during the past five decades. Currently, the cure rate of pediatric ALL is above 80%, comparing with 30% in the late 1960s[2]. These impressive improvements can be attributed to the advances in the understanding of the molecular genetics and pathogenesis of the disease, the incorporation of risk-adapted chemotherapy, and the availability of new targeted therapeutic agents[1]. The risk stratification is commonly adopted in the treatment based on variables of prognostic importance, which include the patient age, initial leukocyte count, immunophenotype, chromosomal aberrations, responsiveness to chemotherapy and minimal residual disease (MRD)[3].

The CCLG-ALL-2008 protocol (the Chinese Children's Leukemia Group-acute lymphoblastic leukemia 08 protocols) was established in 2008 and recommended as a clinical guideline for the treatment of pediatric ALL in China[4]. The treatment regimen contains five phases, including induction, early reinforcement, consolidation, delayed reinforcement, and maintenance treatments[5]. Because MRD techniques by FACS were not available in most children's hospitals in China when the protocol was initially recommended, MRD level was not required in evaluating risk stratification[6]. However, it is well-known in the field that monitoring the level of MRD during chemotherapy is the most important prognostic

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tool in the treatment of patients with ALL[7]. Low MRD after induction chemotherapy is associated with an excellent long-term prognosis whereas patients with high MRD have an extremely poor prognosis[7,8]. Therefore, we have adapted minimal residual disease measurements to guide treatment in the 196 patients enrolled after Nov 2010. In this report, we compared their treatment outcomes with those who were not guided by the levels of MRD in the 153 patients enrolled between Dec 2008 and Oct 2010. Meanwhile, the clinical features, treatment response and long-term survival of entire patient cohort were analyzed.

Patients and Methods

Patients and Treatment Protocol

Between Dec 2008 to Sept 2013, 379 patients aged 3 months to 16 years (median, 4 years) were diagnosed as B-ALL according to MICM criteria in our single institution. Due to the economic burden or disease crisis such as tumor lysis syndrome and life-threating pneumonia, 30 patients didn't proceed with the consolidation therapies and were excluded. As a result, 349 pediatric ALL patients who followed the protocol without hematopoietic stem cell transplantation (HSCT) were calculated for EFS, RFS, and OS. Three hundred and forty-nine patients were analyzed for clinical characteristics, treatment responses, as well as status of remission. Before October 2010, the MRD level wasn't considered as the risk factor and all 153 patients (named as the first cohort) were treated based only on conventional prognostic factors. While the second cohort patients (196 patients) were enrolled in this study after Oct 2010 and MRD level was incorporated into the risk-stratification, these patients were treated on the risk-readapted principle (Table I). Follow-up observations extended through Oct 2014. Details of the risk stratifica-

Table I. Different risk stratification criteria.

	Risk stratification		
	Traditional risk	**MRD-based risk**	**MRD-combined risk**
SR	(all the factors) None of the HR factor; B-precursor ALL; age ranged 1 to 10 years; WBC counts less than 50×10^9/L; the absolute value of spinal fluid blasts < 5×10^6/L; good BM histological remission (M1 or M2) on day 15; histological CR (normal BM cellularity with < 5% undifferentiated cells) achieved on day 33 of induction remission phase.	MRD < 10-4 at TP1	Traditional SR Plus MRD < 10-4 at TP1
IR	None of the HR factor; BM showed M3 after SR induction or M1/M2 after IR induction on day 15; (at least 1 factor) age ≥ 10 years; age < 1 year without MLL rearrangements; WBC counts ≥ 50×10^9/L; the absolute value of spinal fluid blasts >5×10^6/L; T-ALL; E2A-PBX1 fusion gene or t(1;19) chromosomal translocation.	MRD < 10^{-2} at TP1	Traditional IR Plus MRD < 10-2 at TP1
HR	(at least 1 factor) MLL gene rearrangement (t[4;11] chromosomal translocation and/or MLL-AF4 gene fusion or other MLL rearrangement); BCR/ABL fusion gene or t(9;22) chromosomal translocation; early resistance to the 1-week steroid induction (the absolute value of peripheral blasts >1000/μl); poor BM blast clearance (M3) after IR induction on day 15; no histological CR (normal BM cellularity with > 5% undifferentiated cells) achieved on day 33 of induction remission phase	MRD ≥ 10^{-2} at TP1 or MRD ≥ 10^{-3} at TP2	Traditional HR or MRD ≥ 10^{-2} at TP1 or MRD ≥ 10^{-3} at TP2

Abbreviations: SR: standard risk; IR: intermediate risk; HR: high risk; MRD: minimal residual disease; BM: bone marrow; TP1: time point 1, at the end of induction around day 33; TP2: time point 2, before consolidation around week 12; M1:BM cellularity with < 5% undifferentiated cells; M2: BM cellularity with ≥ 5%, but < 25% undifferentiated cells; M3: BM cellularity with ≥ 25% undifferentiated cells.

tion and treatment regimen of the protocol were illustrated in Table II and Figure 1. The CCLG-ALL-2008 treatment protocol was approved by the Children's Hospital of Soochow University Institutional Ethics Committee. Informed consents were signed by the parents or caregivers of each patient.

Cytogenetic Analysis and Fusion Gene Amplification

Conventional cytogenetics analysis was performed at the time of initial diagnosis. Chromosomes were R-banded on BM cells from direct and/or 24-hr stimulated cultures. According to the abnormality of chromosome, three subgroups were classified as the favorable group [t(12;21), hyperdiploid], the intermediate group [t(1;19), t(11;14), t(11;19), del(9p), del(6q), normal chromosome], and the adverse group [t(9;22), t(4;11), hypodiploid]. Meanwhile, the total RNA of each sample was extracted with Trizol (Invitrogen Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. A multiplex reverse transcription polymerase chain reaction (RT-PCR) was performed to identify simultaneously the following fusion transcripts in ALL: BCR-ABL1, E2A-PBX1, TEL-AML1, HOX11 and MLL rearrangements (MLLr).

Minimal Residual Disease (MRD) Analysis

FACS was used for immunophenotypes detection and MRD screening and monitoring. Detection of a leukemia cell was feasible among at least 10000 normal cells (10^{-4}). The MRD level was detected at two-time points, TP1, at the end of induction therapy around day 33 after beginning chemotherapy, and TP2, before intensification around week 12 after finishing consolidation. In this study, MRD information in 327 samples was available at TP1 and TP2 in B-ALL. However, MRD incorporated risk stratification only applied to patients enrolled after Nov. 2010 (n = 196).

Criteria for Risk Evaluation

According to CCLG-ALL-2008 protocol, ALL patients were classified into three hierarchy subgroups as SR, IR, and HR based on the following factors such as, the clinical presentation (age, WBC count, ALL type and gene aberrations), the early treatment response (prednisone response and histological remission status of BM), as well as the MRD levels (Table I). In this study, we separated these factors into conventional risk-stratification, MRD-based

Table II. CCLG-2008 treatment protocol.

CCLG-2008	Prednisone induction	Remission induction	Early intensification	Consolidation	Delay intensification I	Maintenance I	Delay intensification II	Maintenance II
Standard-risk	P (one week)	VDLD (DNR × 2)	CAM	HD-MTX (2 g/m^2) × 4	VDLD+CAM	/	/	6-MP+MTX/VD+IT
Intermediate-risk	P (one week)	VDLD (DNR × 4)	CAM × 2	HD-MTX (5 g/m^2) × 4	VDLD+CAM	6-MP+MTX	VDLD+CAM	6-MP+MTX/VD+TIT
High-risk	P (one week)	VDLD (DNR × 4)	CAM × 2	I'II'III' × 2	VDLD+CAM	/	/	6-MP+MTX/CA/VD+IT

CCLG, Chinese Childhood Leukemia Group; VDLD, vincristine+daunorubicin+L-asparaginase+dexamethasone; CAM, cyclophosphamide +cytarabine+6-mercaptopurine (6-MP); HD-MTX, high-dose methotrexate; VD, vincristine+dexamethasone; IT, intrathecal injection with dexamethasone and methotrexate; CA, cyclophosphamide+cytarabine; TIT, intrathecal injection with dexamethasone, methotrexate and cytarabine; I', Berlin-Frankfurt-Munster (BFM) High Risk block-1';II', BFM High Risk block-2'; III', BFM High Risk block-3'.

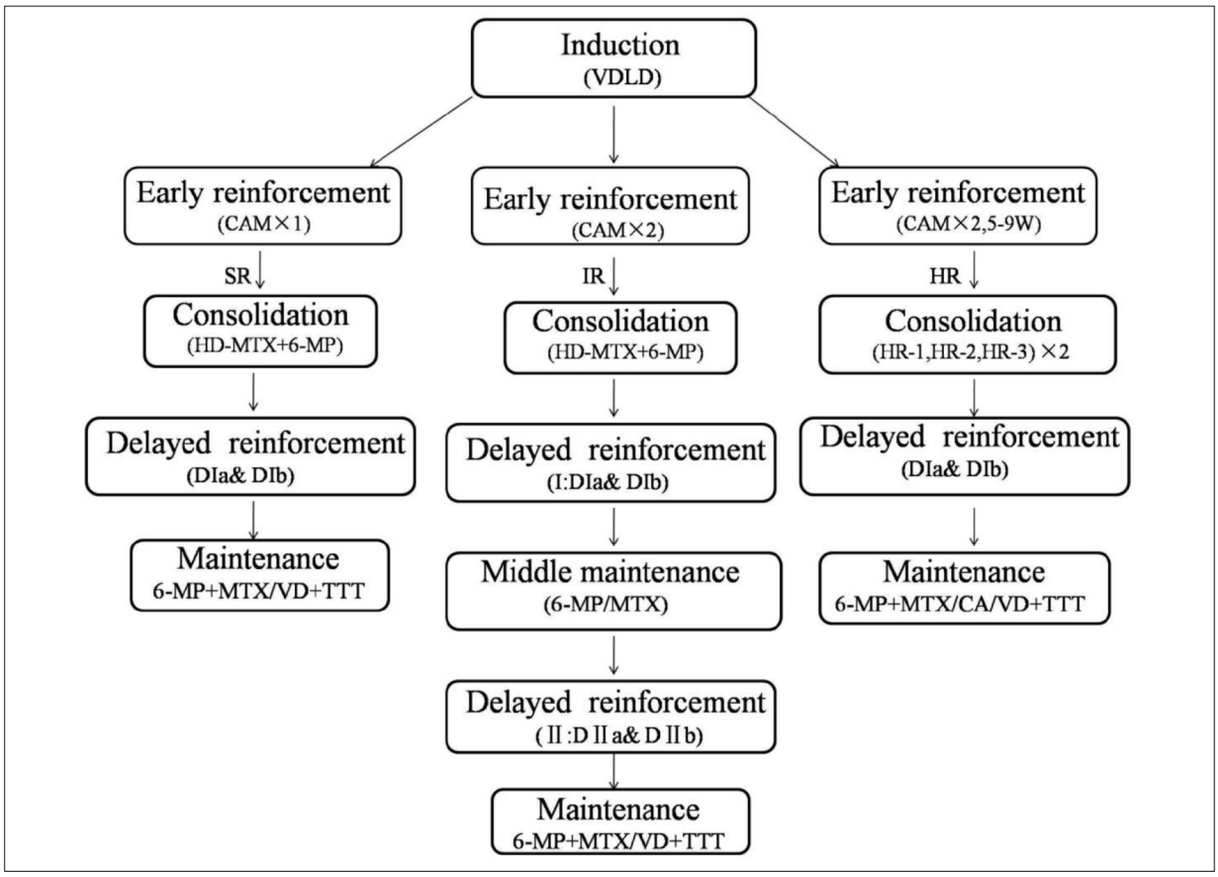

Figure 1. The flow chart of CCLG-2008 treatment protocol. Note: VDLD, vincristine+daunorubicin+L-asparaginase+dexamethasone; CAM, cyclophosphamide +cytarabine+6-mercaptopurine (6-MP); HD-MTX, high-dose methotrexate; VD, vincristine+dexamethasone; IT, intrathecal injection with dexamethasone and methotrexate; CA,cyclophosphamide+cytarabine; TTT, intrathecal injection with dexamethasone, methotrexate and cytarabine; BFM, Berlin-Frankfurt-Munster; HR-1, BFM High Risk Block-1; HR-2, BFM High Risk Block-2; HR-3, BFM High Risk Block-3.

risk stratification and MRD-combined risk-stratification. The detailed information was listed in Table I.

Treatment Response Definitions

Poor response to prednisone (PPR) was defined as the presence of more than 1000 blasts/μL blood blasts on day 8 after prednisone treatment, otherwise, good prednisone response (GPR). Relapse was defined as the reappearance of leukemic cells in BM (> 25% blasts) after CR. CNS relapse was defined as more than 5 blasts/μL in the CFS. Testicular relapse was diagnosed clinically and confirmed with ultrasonography. Very early relapse refers to less than 18 months from the first diagnosis; early relapse refers to 18 months or more after the first diagnosis and less than 6 months from stopping therapy; late relapse refers to 6 months or more after stopping therapy[9].

Statistical Analysis

Data collection ended on October 31th, 2014. RFS was defined as the duration from the time of diagnosis to the date of disease relapse. EFS was defined as the duration from the date of diagnosis to the date of relapse, death, or treatment failure, whatever came first, or the last contacting with patients in continuous hematological complete remission (CR). OS was referred to the date of diagnosis to the date of death or last follow-up. The analysis of RFS, EFS and OS was calculated with the Kaplan-Meier procedure. Comparisons between different classified groups were performed with the log-rank test. The Cox proportional hazards regression model was used to evaluate the significance of differences in survival among the clinical indicators. All tests were two-sided with a $p < 0.05$ considered statistically significant. SPSS 16.0 software (SPSS Inc., Chicago, IL, USA)was used for all statistical analysis.

Results

Clinical Features of ALL Patients

Clinical features and biological characteristics at the time of initial diagnosis were illustrated in Table III. The detailed information on chromosome distribution was listed in Figure 2. The fusion gene transcripts were illustrated in Table III.

Clinical Prognostic Factors and Treatment Response

Table I indicated that patients with WBC counts below 50×10^9/L had a better response to prednisone induction ($p < 0.01$). No gender bias was observed in the responses of the treatment. Pediatric patients older than 10 years showed a poorer response with less histological BM remission on day 15 ($p = 0.048$) than the younger patients. As a result, the relapse rate was higher in the older patients (> 10 years old patient population, $p = 0.002$). The relapse rate was 19.2% (74/379), equally distributed at different stages [7.4% (28/379) very early, 6.3% (24/379) early, and 5.8% (22/379) late stages, respectively, Table III]. Moreover, in most cases the relapse sites were in BM except for five in the testis and two in the central nerves systems. Our data indicated that WBC and age were the most important prognostic factors, which were consistent with previous reports. Relapse is a serious issue that contributes to the poor outcome of the treatment of ALL.

The Influence of Gene Rearrangement on the Treatment Response

In our clinical observation, patients with different types of fusion transcripts had diverse responses to the treatment. Comparing with normal cytogenetic patients, those with *TEL-AML1*$^+$ or *HOX11*+ had no differences in treatment response; among positive fusion transcript cohort, patients with *E2A-PBX1* showed a favorable outcome with lower MRD level (< 10^{-4}) at TP1 ($p = 0.039$) (Table III). On the contrary, patients with *MLL* rearrangements and *BCR-ABL1* fusion gene showed an adverse response. These two groups had a poor response to prednisone pretreatment ($p < 0.001$and 0.015, respectively) (Table III). Meanwhile, the *BCR-ABL1*+ group had a higher level of MRD (> 10^{-2}) at TP1 ($p < 0.001$). Given the cytogenetic aberrations, we found that patients with favorable karyotypes had good prednisone response compared with those with intermediate and adverse ones, with a p-value of 0.013 and < 0.001, respectively. Moreover, patients with favorable karyotypes usually were with the lower level of MRD at TP1 (< 10^{-2}) and TP2 (< 10^{-3}) (Table III). Our results indicated that fusion gene transcripts do play an important role in chemo-response and *E2A/PBX1* positive patients responded well to CCLG-ALL-2008 protocol.

The Effect of Different Indictors on RFS, EFS and OS

Using Cox regression, we found that age, gene rearrangement and MRD-based risk stratification were all independent prognostic factors for RFS, EFS and OS (Table IV). WBC count was an independent prognostic factor of RFS and EFS, prednisone response and remission status of BM after induction treatment were independent prognostic factors of EFS and OS (Table IV). The adverse features were high WBC counts, age ≥10 years, fusion gene transcripts with *BCR-ABL* or *MLL* rearrangement, poor response to prednisone and BM remission status. However, the karyotypes in our study couldn't be characterized as an independent factor for the data analysis (Table IV). Our results confirmed the previously reported predicting factors by other groups using different protocols[10-12].

The Influence of MRD on RFS, EFS and OS

The first cohort (n = 153) were given risk-adapted treatment based on the conventional risk-stratification without the MRD evaluation. They were classified into three groups, including 86 cased in SR, 47 in IR and 20 in HR. The 5-year RFS, EFS and OS was 82.8 ± 4.2%, 82.8 ± 4.2%, and 85.0 ± 4.0% for SR, 70.9±10.5%, 70.9 ± 10.5%, and 85.1 ±5.2% for IR, and 48.2 ± 20.9%, 42.8 ± 18.9%, and 70.0 ± 10.2% for HR, respectively ($p < 0.001$, Figure 3). After Nov 2010, the MRD detection became fully functional, 196 patients were treated according to the evaluation of MRD incorporated with conventional risk factors (the second cohort). Patients were classified into 3 groups, 63 patients in MRD-combined SR, 63 in MRD-combined IR, and 70 in MRD-combined HR. The 5-year RFS, EFS and OS was 88.8 ± 4.9%, 88.8 ± 4.9%, and 94.9 ± 3.8% for MRD-combined SR, 84.7 ± 5.2%, 85.0 ± 5.0%, and 90.9 ± 3.9% for MRD-combined IR, and 35.4 ± 9.4%, 29.2 ± 8.0%, and 33.4 ± 10.3% for MRD-combined HR, respectively ($p < 0.001$, Figure 3). From our data, we

Table III. Clinical characteristics and treatment response of ALL patients.

	Total	Prednisone response			BM remission on day 15				BM remission on day 33			TP1				TP2			Relapse				
		Sensitive	Non sensitive	*p*	M1 < 5%	M2 5%-25%	M3 > 25%	*p*	CR	NCR	*p*	MRD $< 10^{-4}$	MRD $\geq 10^{-4}$, $< 10^{-2}$	MRD $\geq 10^{-2}$	*p*	MRD $< 10^{-3}$	MRD $\geq 10^{-3}$	*p*	Very early	Early	Late	No relapse	*p*
Patients number	379	324	55		202	89	88		352	27		160	130	37		274	53		28	24	22	305	
WBC ($\times 10^9$/L)				**< 0.001**				0.569			0.28				0.226			0.101					0.195
< 50	284	263	21		158	65	61		267	17		129	106	25		219	35		16	16	16	236	
50-100	48	35	13		23	12	13		44	4		18	17	6		34	9		4	3	3	38	
> 100	47	26	21		21	12	14		41	6		13	7	6		21	9		8	5	3	31	
Gender				0.300				0.340			0.426				0.826			0.307					0.874
Female	155	136	19		83	41	31		142	13		65	51	13		103	24		13	11	9	122	
Male	224	188	36		119	48	57		210	14		95	79	24		171	29		15	13	13	183	
Age				0.129				0.048			0.476				0.121			0.230					0.002
≥ 10 years	40	31	9		15	10	15		31	4		9	15	5		23	8		9	4	4	23	
< 10 years	339	293	46		187	79	73		316	23		151	115	32		251	45		19	20	18	282	
Fusion gene				**0.004**				0.161			0.094				**0.002**			0.127					**0.034**
TEL-AML1	61	55	6		34	10	17		59	2		30	20	8		46	9		1	6	2	52	
E2A-PBX1	20	17	3		15	3	2		20	0		13	5	0		18	0		1	0	0	19	
MLLr	18	12	6		10	5	3		17	1		8	13	2		9	3		3	0	1	14	
BCR-ABL1	10	6	4		3	2	5		7	3		2	8	6		3	2		1	0	0	9	
HOX11	20	17	3		15	2	3		19	2		9	2	3		17	3		0	0	1	19	
Undetected	250	230	30	0.004	124	67	59		227	23		98	93	18		167	29		20	18	18	188	
Karyotype				**0.003**				0.198			0.059				**0. 036**			**0. 023**					0.254
Favorable	220	185	35		117	51	52		201	19		86	73	25		140	34		20	14	14	172	
Intermediate	92	87	5		48	29	15		90	2		50	37	3		77	6		3	5	7	77	
Adverse	18	12	6		6	7	5		16	2		3	5	2		7	2		3	5	0	14	

Abbreviations: B-ALL: B-acute lymphoblastic leukemia, WBC: white blood cell, BM: bone marrow, MRD: minimal residual disease, TP1: time point 1: at the end of remission induction around day 33, TP2: time point 2: before consolidation around week 12, CR: complete remission: normal BM cellularity with < 5% undifferentiated cells, NCR: non-complete remission: BM cellularity with ≥ 5% undifferentiated cells, M1: BM cellularity with < 5% undifferentiated cells; M2: BM cellularity with ≥ 5%, but < 25% undifferentiated cells; M3: BM cellularity with ≥ 25% undifferentiated cells. *Note:* Favorable included t(12;21), hyperdiploid; Intermediate included t(1;19), t(11;14), t(11;19), del(9p), del(6q) and normal chromosome; Adverse included t(9;22), t(4;11), hypodiploid. Undetected: samples were not identified fusion gene with the method of multiplex RT-PCR.

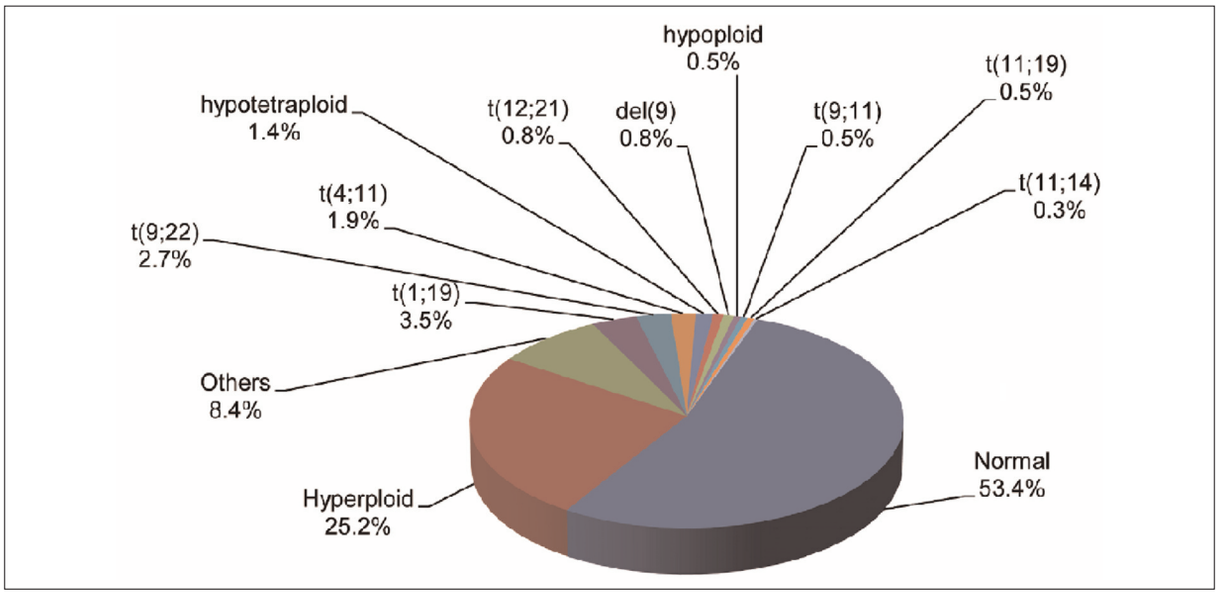

Figure 2. Distributions of different recurring chromosome aberrations in BP-ALL patients.

found that the proportion of HR patients significantly increased as defined by MRD incorporated and had a worse outcome than the first cohort. The SR and IR patients had a better outcome in the second cohort than the first cohort.

We retrospectively reevaluated the outcome of two cohorts with MRD data together to figure out the effect of the MRD level on the patient outcomes without considering the other risk factors. We reclassified the patients into 4 groups and found that the patients with MRD levels less than 10^{-4} had similar OS to the patients with MRD levels less than10^{-3}, patients with MRD levels above 10^{-2} had the worst outcome, and patients with MRD levels higher than $10^{-3}$and lower than10^{-2} had an intermediate outcome at TP2 (Figure 4). From these results, we concluded that MRD alone has the highest correlation in evaluating the effect of the protocol. We propose that the risk levels for MRD to be setup at two levels, namely, 10^{-3} and 10^{-2} based on CCLG ALL 2008 protocol. It is unnecessary to defined MRD level at 10^{-4} as the low risk recommended in the protocol (Table I). In this way, MRD monitor will be easier to operate and promote in most institutions because it is much easier to detect 10^{-3} level than 10^{-4} level for the FACS technicians with the current technology.

MRD-combined HR had more disease relapses than MRD-combined SR ($p < 0.001$) and MRD-combined IR ($p = 0.002$). However, with the traditional risk stratification, there were no significant differences among them of relapse (Table V). Our data indicated that HR patients identified by MRD responded poorly to chemotherapy who are candidates for HSCT to improve their outcome.

Discussion

In the pediatric population, ALL is the most common type of childhood leukemia for patients younger than 15 years, accounting for 26% of all cancers and 78% of leukemia in this age group, and for approximately 20% of adult acute leukemia[13]. Recent advances in molecular biology and clinical studies have led to a wealth of information and deeper understanding of the pathology of ALL. The genetic basis in ALL is of most importance. Till now, more than 50 recurring genetic alterations have been identified, and many of the genes involved encode proteins with key roles in cooperation to leukemia genesis[14]. The incidence rate of distinct fusion transcripts detected in our patients' cohort was 34.0%, which was similar to Gao et al[15] report (37.05%).

Risk-stratification by MRD assessment has already brought about considerable improvement in individualized treatment planning[2]. MRD level has become a highly reliable prog-

Table IV. Variable prognostic indictors of RFS, EFS and OS.

	RFS				EFS				OS			
	p-value	Exp(B)	95.0% CI for Exp(B) low	5.0% CI for 9 Exp(B) up	*p*-value	Exp(B)	95.0% CI for Exp(B) low	95.0% CI for Exp(B) up	*p*-value	Exp(B)	95.0% CI for Exp(B) low	95.0% CI for Exp(B) up
WBC	**0.001**	1.943	1.324	2.851	**0.000**	1.913	1.341	2.728	0.080	1.456	0.956	2.219
Age	**0.001**	2.917	1.545	5.507	**0.002**	2.662	1.450	4.888	**0.001**	3.186	1.621	6.260
Gene aberrations	**0.015**	0.757	0.604	0.948	**0.003**	0.755	0.627	0.911	**0.018**	0.769	0.619	0.956
Karyotypes	0.650	1.080	0.774	1.507	0.578	1.092	0.800	1.491	0.244	1.256	0.856	1.842
Prednisone response	0.126	0.560	0.267	1.176	**0.008**	0.418	0.220	0.793	**0.004**	0.332	0.157	0.702
BM remission status	0.056	2.174	0.981	4.815	**0.000**	3.423	2.078	5.639	**0.000**	3.481	1.997	6.066
MRD-based risk stratification	**0.015**	1.574	1.093	2.267	**0.006**	1.631	1.151	2.310	**0.027**	1.603	1.056	2.433

Abbreviations: RFS: relapse-free survival; EFS: event-free survival; OS: overall survival; WBC: white blood cell; BM: bone marrow; MRD: minimal residual disease.

nostic indicator because it not only reflects intrinsic drug sensitivity, but also treatment adherence, and treatment efficacy[16]. We selected TP1 and TP2 as the check points for MRD measurement, which was consistent with most reports[2,15]. At TP1, the MRD level was higher in groups with age ≥ 10 years, BCR/ABL fusion gene, as well as adverse karyotypes; and at TP2, the MRD level was higher in groups with WBC ≥ 50 × 10^9/L, MLL rearrangements and adverse karyotypes. In our study, patients who received treatment after MRD incorporated for monitoring have improved survival outcomes of SR and IR comparing with patients received treatment without MRD monitoring. The risk of relapse was more in the MRD-based HR patients who are candidates for HSCT to improve their outcomes.

The univariate survival analysis demonstrated that the MRD measurements at both time points had prognostic significance to 5-year RFS, EFS, and OS. The risk was consistent with MRD level. Gao et al[15] reported that higher MRD levels at the end of induction and before intensification were related to elevated WBC counts and a poor prednisone response. Our results demonstrated that MRD is a validated early index of the response to treatment, an important biologic feature in risk stratification and modification of treatment intensity at an early phase. Patients with MRD level above 1% should be considered as candidates for HSCT after entering intensification instead of chemotherapy alone. In this study, we also found that patients with MRD level at 10^{-4} showed a similar outcome to patients with MRD level at 10^{-3} treated by the protocol. It indicated that the cut-off level of MRD at 10^{-3} was a reasonable value for a risk-stratified treatment. This finding is very useful and helpful in China because the CCLG-ALL-2008 protocol has been recommended as the clinical guideline for Chinese pediatric ALL. In practice, detection of MRD level at 10^{-3} is much easier and more promote in operation than the 10^{-4} level for typical labs in China.

The 5-year EFS and OS in our study were less than those reported from the western countries[16-18], but similar to those from Asian countries[12,19,20]. This could be resulted from the less intensive treatment because of the poor economic situation in some families and early death due to insufficient supportive cares. The relapse rate in our study was 17.9%, a little higher than 15% reported in literature[21]. A large proportion of relapse arose from bone

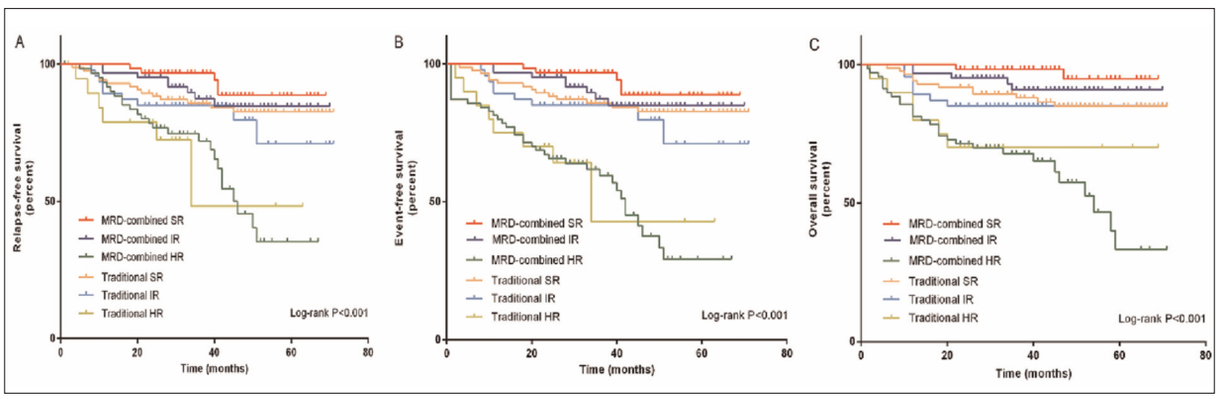

Figure 3. Outcome of two cohorts. ***(A)*** The 5 years RFS of two cohorts. ***(B)*** The 5 years EFS of two cohorts. ***(C)*** The 5 years OS of two cohorts.

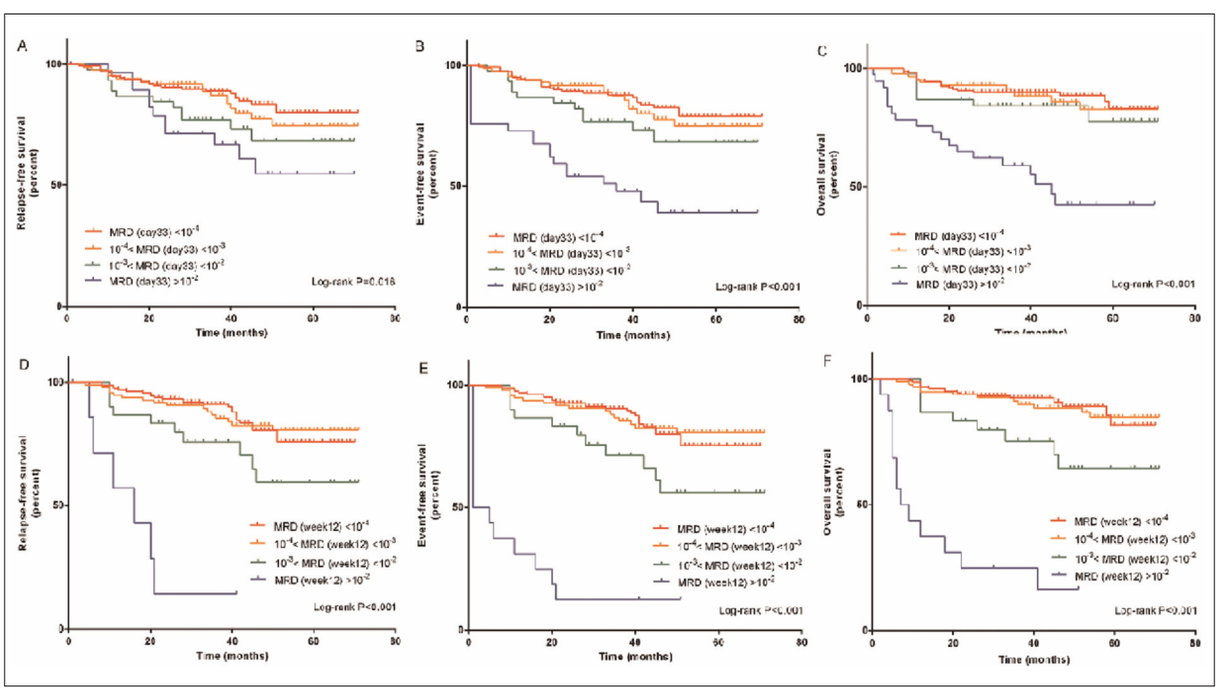

Figure 4. The outcome of patients classified only based on MRD level. ***(A)*** Comparison of the five years RFS among different levels of MRD at TP1. ***(B)*** Comparison of the five years EFS among different levels of MRD at TP1. ***(C)*** Comparison of the five years OS among different levels of MRD at TP1. ***(D)*** Comparison of the five years RFS among different levels of MRD at TP2. ***(E)*** Comparison of the five years EFS among different levels of MRD at TP2. ***(F)*** Comparison of the five years OS among different levels of MRD at TP2. *Note:* TP1 indicated the day 33 after induction therapy started. TP2 indicated the time around 12 week before consolidation therapy started.

Table V. Relapses in groups of MRD based risk stratification or MRD uncombined.

	Traditional risk stratification group				MRD-combined risk stratification group			
	SR	IR	HR	*p*-value	SR	IR	HR	*p*-value
Relapse	14	9	6	0.370	5	8	25	< 0.001
Non-relapse	72	38	14		58	55	45	

Abbreviations: MRD: minimal residual disease; SR: standard risk; IR: intermediate risk; HR: high risk.

marrow. Attributed to the intensification of prophylactic intrathecal chemotherapy of CCLG-ALL-2008 protocol, the recurrence in the center never systems were rare in our study. Nowadays, the effective systemic and intrathecal therapy had opened an exciting way for eliminating prophylactic cranial irradiations[16].

Conclusions

Our prospective study proved that MRD monitoring during remission induction treatment and before intensification treatment had important prognostic and therapeutic implications which could be incorporated into the risk factors in the protocol. Patients with high MRD levels either at TP1 or TP2 should be considered as HSCT candidates to improve their outcomes in the long run.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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