Minimal Residual Disease in Pediatric Leukemia

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The prognosis for pediatric leukemia, especially for acute lymphoblastic leukemia (ALL), which is most popular in pediatric leukemia, has improved, and many patients are expected to be cured. On the other hand, there are not a few refractory and relapsed patients predicted poor prognosis, so it is important to find risk factors for prognosis at an early stage and treat according to these risk factors. As risk stratification, treatment response in addition to the phenotype and genotype of leukemic blasts has been used. Recently, much attention has been focused on minimal residual disease (MRD) as treatment response and prognostic factor. The MRD means detection of "deepness of complete remission" and the detection of MRD involves searching for chimeric transcripts, or specific gene abnormalities by polymerase chain reaction (PCR) and/or specific surface antigens by flow cytometry (FCM) of residual leukemic cells at morphological remission. In this lecture, current status and future directions of MRD study for pediatric leukemia with history of clinical trials are presented.

Key Words : Leukemia, Children, MRD

Introduction

Recently, many patients with pediatric leukemia—especially acute lymphoblastic leukemia (ALL), which is the most common leukemia in children—have become curable, whereas not a few patients remain refractory and relapse-prone. For such cases, early detection and provision of stratified treatments according to their estimated prognoses are important. As risk stratification factors, treatment response has been employed in addition to the phenotype and genotype of leukemia, and minimal residual disease (MRD) has recently attracted a considerable amount of attention as an indicator of treatment response. MRD refers to the "depth of complete remission (CR)" at the time of morphological CR and can be quantified mainly *via* multicolor flow cytometry (FCM) or polymerase chain reaction (PCR) based on chimeric transcripts or leukemic cell-specific abnormalities. This lecture presents the current status and future prospects of MRD studies in pediatric leukemia, along with historical transitions in pediatric leukemia treatment.

Pediatric leukemia

Leukemia is the most common cancer in children. Unlike adult cases, ALL accounts for the largest proportion of all childhood leukemias, with approximately 450 to 500 new cases reported annually in Japan. Acute myeloid leukemia (AML) is the second most common, with roughly 150 to 200 new annual cases in Japan, as opposed to chronic myeloid leukemia, which is extremely rare in children with only 20 new cases or so diagnosed annually in the same country. As for chronic lymphoblastic leukemia or myeloma, no

pediatric patients have been reported.

Although relatively common among pediatric diseases, leukemia is still an infrequent condition. Multicenter therapeutic trials have been conducted by joint study groups both in and outside of Japan to achieve better understanding and treatment of this disease. In Japan, group studies on pediatric leukemia across the nation led to the founding of the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) in 2003, which then evolved into the Japan Children's Cancer Group (JCCG). Currently, almost all clinical trials for childhood leukemia are conducted by the JCCG in an All-Japan framework.

Characteristics of pediatric ALL

ALL constitutes approximately 70% of cases of pediatric leukemia. It can develop at any age but most frequently between ages 2 and 5 years. Multiagent chemotherapy (chemo) including central nervous system (CNS) prophylaxis is provided. Recent treatment outcomes for pediatric ALL have improved, achieving > 80% long-term survival rates. This accomplishment is largely attributed to better understanding of the disease, treatments developed based on the upgraded understanding, risk stratification according to prognostic factors, and progress in maintenance therapy. For the reduction of short- and long-term therapy-related toxicities along with the improvement of the overall treatment outcomes, prognostic factor-based risk stratification is essential. In patients at lower risk for relapse, how to perform treatment reduction is another important challenge.

Prognostic factors differ depending on treatments. The age and peripheral blood white blood cell count at the initial diagnosis are called classical prognostic factors and are still currently used; however, their value is shrinking with the discovery of various new factors. **Fig 1** presents a treatment algorithm for pediatric ALL provided in "A practical guideline for pediatric leukemia and lymphoma 2016"[1]. In addition to the factors obtained at the time of diagnosis (e.g., the age, white blood cell count, CNS/testicular involvement, immunoprofiling of blasts, chromosomal/gene abnormalities), early therapy response and CR are adopted as the risk stratification factors. Mainstay treatment is the conventional chemo, whereas tyrosine kinase inhibitors (TKIs) are added to chemo regimens to treat Philadelphia chromosome-positive ALL (Ph+ ALL). For poor responders or patients with an unfavorable prognostic factors, an allogeneic hematopoietic stem cell transplant (allo HSCT) may be indicated. Additional recent possible options include blinatumomab or chimeric antigen receptor T-cell (CAR-T) therapy targeting CD19 mainly for relapsed ALL, and Inotuzumab Ozogamicin (InO) treatment targeting CD22 that is not currently used to treat pediatric cases. As treatment response indicators, in addition to morphologic evaluation results, such as CR, response to prednisolone monotherapy (number of blasts in peripheral blood on treatment day 8) during remission induction therapy and MRD positivity/negativity in CR are used.

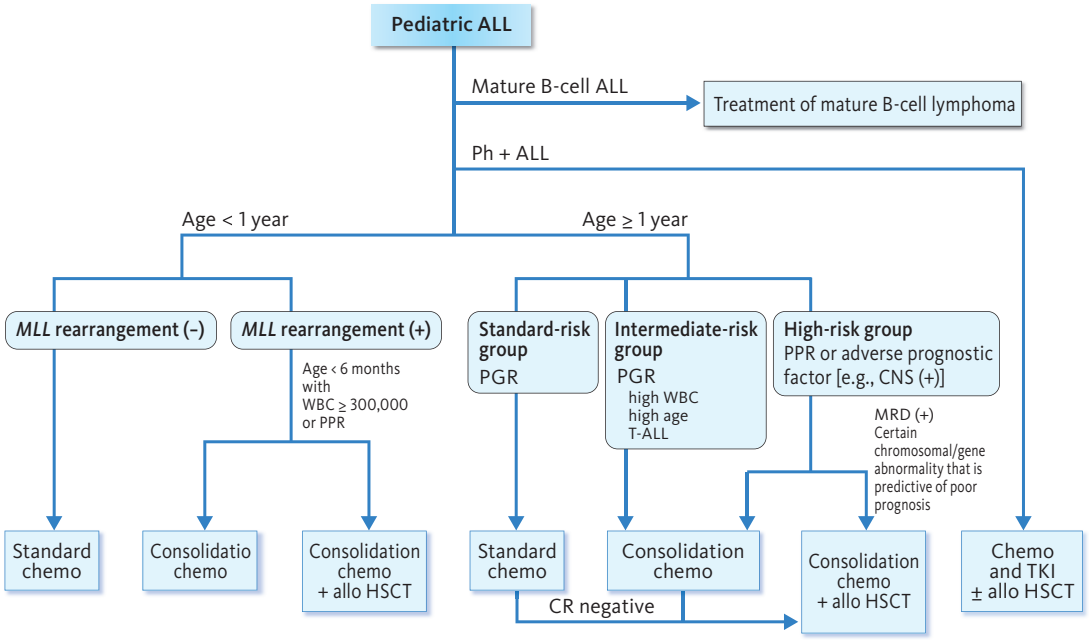

Fig 1. Treatment algorithm for pediatric ALL

(Excerpt from "A practical guideline for pediatric leukemia and lymphoma 2016" edited by the Japanese Society of Pediatric Hematology/Oncology)

MLL, mixed-lineage leukemia; PPR, prednisolone poor responder; PGR, prednisolone good responder; T-ALL, T-cell acute lymphoblastic leukemia

Characteristics of pediatric AML

AML accounts for roughly 25% of the pediatric leukemias, being the second most common next to ALL. As shown in the treatment algorithm for pediatric AML (**Fig 2**) from "A practical guideline for pediatric leukemia and lymphoma 2016", three subtypes are separately treated: acute promyelocytic leukemia (APL), AML associated with Down Syndrome (ML-DS), and *de novo* AML (i.e., primary AML other than APL or ML-DS)[1]. For APL, although caution is needed for possible disseminated intravascular coagulation at the time of the disease onset, survival rates have improved to over 90% with the use of all-trans retinoic acid (ATRA), chemo, or, recently, arsenic trioxide. ML-DS is characterized by early-onset age, a low percentage of circulating blasts at the time of onset, and megakaryoblastic feature, among others. Generally, patients with ML-DS respond well to treatment but tend to develop treatment-related toxicities; thus, ML-DS is treated with therapy for AML without DS at reduced intensity, achieving high survival rates of approximately 90%. For *de novo* AML, risk stratification is based on cell biological features, such as blast chromosomal and chimeric gene profiles, and treatment

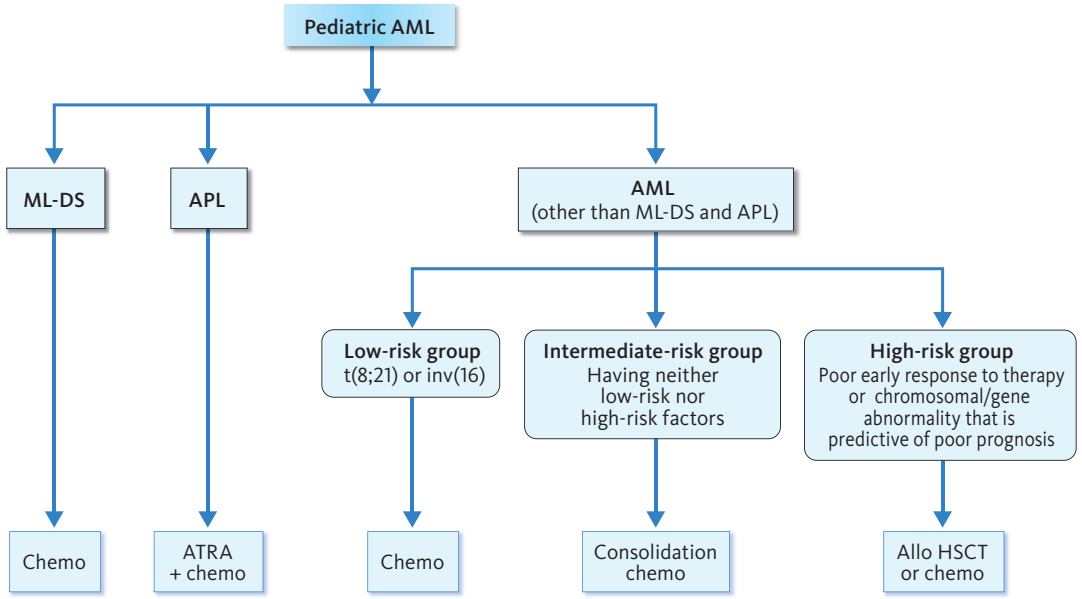

Fig 2. Treatment algorithm for pediatric AML

(Excerpt from "A practical guideline for pediatric leukemia and lymphoma 2016" edited by the Japanese Society of Pediatric Hematology/Oncology)

response. The poor prognosis group (high-risk group) patients are often indicated for an allo HSCT during the initial CR.

In clinical trials, both domestic and overseas, the percentage of malignant blasts remaining in the bone marrow after the induction therapy is commonly evaluated morphologically as a treatment response indicator. In the AML-05 trial by the JPLSG (currently JCCG) and the AML-12 trial by the JCCG, post-induction hematological CR-negative patients were evaluated as having poor prognosis and were thus indicated for allo HSCT, even in those who subsequently achieved the CR[2].

MRD studies in pediatric ALL

In pediatric ALL clinical trials conducted in the late 1990s, FCM- and PCR-based retrospective analyses demonstrated the MRD levels at the end of induction therapy and at other time points in the treatment course to be powerful predictors of relapse[3,4]. Subsequently, MRD was established as a prognostic factor, and an increase in therapy intensity for high-risk MRD-positive patients was reported to improve their event-free survival rates (EFS)[5,6]. On the other hand, in low-risk patients defined by the status of MRD, treatment outcomes were maintained after reducing the therapy intensity, indicating

the potential of MRD as a basis for selecting appropriate treatment intensity[7]. Research also advanced in relapsed ALL and allo HSCT cases: for the former, the utility of allo HSCT in MRD-positive cases after the second course of induction therapy[8], and for the latter, differences in the prognosis depending on the MRD levels before and after allo HSCT[9] were reported.

Currently, stratified treatment of childhood ALL is using mainly FCM (FCM-MRD) in America, and Ig/TCR PCR-MRD in Europe as a standard. In Japan, the Ig/TCR PCR-MRD assessment became covered by public health insurance in April 2018 and has been in use for treatment stratification.

The FCM-MRD method is capable of rapidly detecting MRD at the sensitivity of 0.01%, whereas it has shortcomings of being inferior in detection sensitivity to the Ig/TCR PCR-MRD method and has difficulty in assessing MRD in cases of protein surface antigen shifting, which can occur in patients with ALL relapse after treatment, especially recently introduced CD19-/CD22-targeted immunotherapy or CAR T-cell therapy. On the other hand, whereas the Ig/TCR PCR method has a MRD detection sensitivity that is 10 times higher (0.001%) than that of the FCR-MRD method, the procedure is laborious to a degree (e.g., designing specific primers for each case required), and MRD evaluation becomes difficult in the event of treatment-induced clonal evolution. In recent years, a next-generation sequencing method with Ig/TCR PCR for MRD quantification has become available, which has even a higher sensitivity and can address clonal evolution. This new method has shown its clinical significance in childhood ALL[10].

MRD studies in pediatric AML

Following the example of the studies conducted in the late 1990s to evaluate the clinical significance of MRD in pediatric ALL, studies on childhood AML have also been conducted in the 2000s and thereafter by the US and European research groups. **Table 1** summarizes the major MRD studies in childhood AML conducted to date[11–17].

In its AAML03P1 trial, the Children's Oncology Group (COG) of the USA assessed FCM-MRD at the end of each induction therapy and found significantly higher relapse rates in MRD-positive (MRD levels ≥ 0.1%) patients than in MRD-negative ones at the end of the first and second inductions and at the end of the treatment. The multivariate analysis including cytogenetic and molecular factors demonstrated FCM-MRD positivity to be an independent predictor of poor prognosis[13].

The AML02 trial in patients aged 21 or less with childhood AML conducted by St. Jude Children's Research Hospital (USA) reported significantly higher relapse or induction failure rates in FCR-MRD-positive patients (MRD ≥ 0.1%) after the first induction course (Ind1). This held true only in the high-risk group and not in either the low- or standard-risk group. The relapse and induction failure rates were significantly higher in patients with MRD ≥ 1% than in those with MRD at 0.1% to < 1%. After induction 2, as in post-induction 1 cases, MRD positivity was significantly associated

Table 1. MRD studies in pediatric AML

Study group	Cases (n)	Method	Measurement time points	MRD cutoffs	Results	Reference
BFM (Germany)	150	FCM	Ind 1	<0.1% 0.1–1% >1%	3EFS: 71% vs 48%	11)
DCOG (Netherlands)	94	FCM	Ind 1, 2, Cos, End of treatment	<0.1% 0.1–0.5% >0.5%	Ind 1; 3RFS: 85% vs 64% vs 13%	12)
COG (USA)	188	FCM	Ind 1, 2, End of treatment	<0% 0–1%	Ind 1; RFS: 65% vs 30% Ind 2; 65% vs 29%, EOT; 62% vs 17%,	13)
St. Jude (USA)	203	FCM	Ind 1, 2, End of treatment	<0.1% 0.1–1% >1%	Ind 1; 3EFS: 74% vs 43%, Ind 2; 71% vs 36%	14)
AIEOP (Italy)	142	FCM	Ind 1, 2	<0.1% 0.1–1% >1%	Ind 1; 3DFS: 73% vs 38% vs 34%	15)
AIEOP (Italy)	49	*RUNX-RUNXIT1*	Ind 2	>2 log reduction	6OS: 86% vs 58%	16)
TCCSG (Japan)	34	FCM	Ind 1, 2	< 0.1% 0.1–1% >1%	Ind 1; 3EFS: 83% vs 33%, Ind 2; 77% vs 20%	17)

Ind1: after the first induction therapy; Ind2: after the second induction therapy; EFS: event-free survival (the preceding number refers to years of survival; likewise below); OS: overall survival; DFS: disease-free survival; and RFS: relapse-free survival.

BFM, Berlin-Frankfurt-Münster Group; DCOG, Dutch Childhood Oncology Group; TCCSG, Tokyo Children's Cancer Study Group

with a higher incidence of relapse and induction failure, but the MRD level-based differences in the incidence rate were not observed. The multivariate analysis found that MRD value more or equal 1% post-Ind1 and non-core binding factor (CBF) AML were significant predictors of inferior event-free survival, and that post-Ind1 MRD≥ 1% plus age 10 and older, 11q23 translocations besides t(9;11), and FAB M7 besides t(1;22) were significant predictors of poor overall survival (OS)[14].

Italy's Associazione Italiana di EmatoOncologia Pediatrica (AIEOP)-AML 2002/01 trial reported significantly lower disease-free survival (DFS) rates in morphologically CR cases with FCM-MRD ≥ 0.1% than in those with FCM-MRD < 0.1% after the first

induction and also after the second induction. The multivariate analysis found post-Ind1 MRD, as well as monosomal karyotype, to be a significant predictor of poor prognosis[15]. In similar AIEOP trial, *RUNX1-RUNX1T1* and *CBFB-MYH11* fusion transcripts were measured with RQ-PCR and at the time of diagnosis and after induction therapy and retrospectively analyzed. ≥ 2 log reduction in the *RUNX1-RUNX1T1* fusion transcript copies after the second induction was associated with significantly favorable prognosis[16]. Japan's JPLSG AML-12 trial measured post-induction FCM-MRD, and evaluation of its significance is currently ongoing[2].

Evaluation of the significance of MRD in the treatment of ML-DS in Japan (JPLSG AML-D11 trial)

As previously stated, childhood AML is commonly divided into three categories, namely, APL, ML-DS, and others (i.e., *de novo* AML), and these are separately treated[1]. ML-DS displays characteristics that are different from those of AML in children without DS, namely, almost always early onset in the first 4 years of life, a large majority of cases being acute megakaryoblastic leukemia, acquired *GATA1* mutations in almost all blasts, leukemic cells' high drug susceptibility, high frequency of treatment-related toxicities, and so on. Given those, ML-DS has been treated separately from ML of non-DS both in Japan and abroad using reduced intensity therapy[18–21]. Compared with other countries, researchers in Japan have been using less intense treatment and lower cumulative dose of a key drug cytosine arabinoside (Ara-C) in therapeutic trials, and have achieved favorable outcomes, with fewer treatment-related mortalities. On the other hand, our retrospective analysis of relapsed/refractory ML-DS cases has demonstrated that they are hard to manage even with allo HSCT and have very poor prognoses[22]. Currently, it is difficult to identify patients with unfavorable prognoses on the basis of disease information available at initial diagnoses and morphological treatment response levels. Appropriate risk stratification methods and salvage regimens for patients with poor prognoses are awaited to be developed. Against such a backdrop, the JPLSG AML-D11 trial was conducted to evaluate MRD, which is a promising prognostic factor, due to its assessment feasibility and effectiveness[23]. Following the treatment components of a predecessor study JPLSG AML-D05, FCM-MRD and MRD measured by *GATA1* mutation-targeted deep sequencing (*GATA1*-MRD) after induction therapy and at the end of the treatment, and the feasibility of MRD measurement and the utility of MRD as a factor for risk stratification were evaluated. Post-induction FCM-MRD and *GATA1*-MRD positivities in the standard-risk group patients were significantly associated with poor prognosis (**Fig 3**). FCM-MRD and *GATA1*-MRD findings were well correlated.

On the basis of the above results, a therapeutic trial JCCG AML-D16 (jRCTs041190047) was initiated in July 2019, in which treatment reduction for post-induction FCM-MRD-negative cases was evaluated.

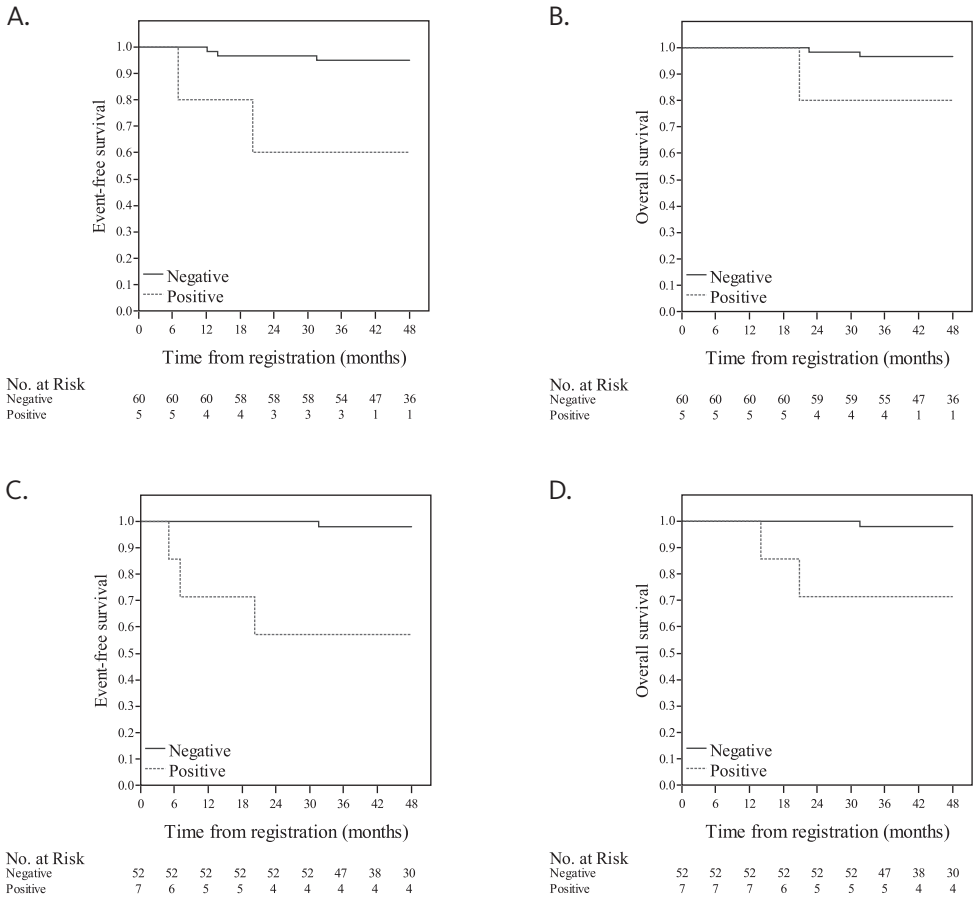

Fig 3. Survival rates based on FCM-MRD and *GATA1*-MRD in the JPLSG AML-D11 trial

A: DFS based on FCM-MRD positivity/negativity, B: OS based on FCM-MRD positivity/negativity,
C: DFS based on *GATA1*-MRD positivity/negativity, D: OS based on *GATA1*-MRD positivity/negativity

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Future prospects

The value of MRD as a prognostic factor is becoming increasingly evident, similar to biomarkers at diagnosis; however, since prognostic factors differ depending on individual treatment regimens, caution is required in the evaluation of prognostic factor-based estimates obtained in each case. Many challenges remain to be addressed, including the standardization of MRD measurement methods and how to manage MRD-positive cases. For these aspects, verification is needed in prospective studies involving a sufficiently large number of patients.

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