Children Diagnosed as Mixed-Phenotype Acute Leukemia Didn't Benefit from the CCLG-2008 Protocol, Retrospective Analysis from Single Center

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Abstract Mixed phenotype acute leukemia (MPAL) is a rare type of acute leukemia with a poor clinical outcome which lacks specific therapy. To evaluate the therapeutic efficiency of CCLG-2008 protocol used for acute lymphoblastic leukemia (ALL) in China on MPAL children who were initially diagnosed as ALL by morphology, we reviewed patients' database diagnosed as ALL and MPAL according to WHO classification and compared their outcomes from July 2008 to June 2012. Total newly enrolled ALL in this study were 309 cases by morphology, in which ten cases were identified as MPAL mainly by immunophenotyping: B+ myeloid (3/10), T+ myeloid (2/10), B + T (4/10), trilineage (1/10). Two cases were classified as intermediate risk (IR) and 8 cases were high risk (HR) according to the CCLG-2008 criteria. Only one case of IR survived and others died due to primary resistance of chemotherapy and relapse. Compared with MPAL, ALL children in IR and HR had a longer survival (28.1 vs 9.5 months, $p < 0.0001$) and lower relapse (16.3 vs 85.7 %, $p = 0.0002$). In a summary, our result indicated

that MPAL in children is a poor-risk disease which needs personalized therapy to improve outcome.

Keywords Mixed-phenotype acute leukemia (MPAL) · Acute lymphoblastic leukemia (ALL) · Children · CCLG-2008 protocol

Introduction

As MPAL represent only 3–5 % of acute leukemias occurring in patients of all ages and comprise several different subtypes (biphenotypic, bilineal, and lineage switch), the optimal therapeutic approach to these cases in pediatric patients has not been well established. Several reports suggested that patients with MPAL have poor treatment outcomes [1–5]. The low frequency of MPAL in pediatric patients makes it difficult to discover clinical insights of MPAL and the best therapeutic approach for these patients. Here we assess the pathologic and clinical features in 10 consecutive cases of pediatric MPAL from 309 acute lymphoblastic leukemia (ALL) which were treated with CCLG-2008 protocol from July 2008 to June 2012 in our hospital.

Materials and Methods

Patients

All the patients diagnosed as ALL were enrolled in this study from July 2008 to June 2012 at the Children's Hospital of Soochow University. The diagnosis and classification of ALL relies on a multidisciplinary approach including morphology, immunophenotyping, karyotyping and more specific molecular genetic analysis. MPAL was

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identified according to the recent WHO criteria [6] from ALL. All the patients were given CCLG-2008 protocol for their initial diagnosis of ALL which was divided into five phases: remission induction (VDLD), early reinforcement (CAM), consolidation therapy, delayed reinforcement (DIa & DIb) and maintenance treatment (Supplemental Figure, SF).

Hematopathology

Morphological type was determined by French-American-British (FAB) criteria, and cases were classified as ALL.

Flow cytometric (FCM) immunophenotyping was performed on bone marrow specimens after lysis according to standard techniques. FCM analysis (four-color) was performed on blast cell populations identified by CD45 versus light side-scatter properties (BD Biosciences, San Jose, CA), standard staining and analytic methods. All cases were characterized with a panel of antibodies to leukocyte-associated markers. In EGIL scoring system surface antigen expression were considered positive if at least 20 % of blasts showed a positive labeling. For cytoplasmic antigen expression, the threshold was 10 %. For anti-MPO more than 5 % of blasts showing a positive labeling could be considered positive.

Cytogenetics Studies

Cytogenetic studies were performed on BM samples by using standard banding techniques, as previously described [7].

Fusion-Gene Detected by Multiple Semiquantitative RT-PCR

Diagnosing of ALL patients was simultaneously detected for 13 types of fusion genes products by multiple semiquantitative RT-PCR in order to find positive fusion gene as a marker for MRD monitoring and evaluation. The procedure/technique was previously published by our group [8].

Statistical Methods

Contingency tables and the Chi square test were used to assess the associations between clinical and laboratory variables. Survival curves were calculated by the Kaplan–Meier method.

Results

Patients Characteristics

There were 309 newly diagnosed cases of ALL enrolled in this study between Jul. 2008 and Jun. 2012 in our single

center. 299 cases of ALL were classified into three risk groups, namely, 118 cases with low risk (LR), 97 cases with intermediate risk (IR) and 84 cases with high risk (HR) according to the multiparameters. Ten cases were identified as MPAL from ALL by immunophenotyping although the morphology was consistent with ALL, namely 3 cases for B+ myeloid, 2 for T+ myeloid, 4 for B + T, and one case for trilineage (Table 1). Cytogenetics evidenced t(9;22)/Ph + (1/10), aberrant (5/10), and normal (3/10) karyotypes (Table 2). Fusion gene disclosed BCR/ABL combining with HOX11 positive (2/10), dupMLL (1/10) (Table 2). Two cases were classified in IR and 8 were in HR (Table 3). There was no case with LR. All the patients were treated by the risk-based strategy therapy of CCLG-2008 protocol (SF), in which bone marrow transplantation (BMT) was an optional therapy for those patients who didn't respond well to chemotherapy [9].

Response to Therapy and Outcome

One case abandoned at initial stage and was excluded from the further study. Nine cases of MPAL received induction therapy of ALL corresponding to IR and HR, respectively. Considering CR, only two cases of MPAL with IR got CR, four cases with HR got CR after the first CAM, namely, Cyclophosphamide, Thioguanine, and Cytarabine, one case with HR got CR after second CAM, two cases with HR didn't get CR and gave up in consolidation therapy (Table 3). These patients who got failure after remission therapy didn't change therapy because their parents didn't take more risk at the most cost after informed poor outcome after failure to induction therapy considering their family economic status. CR rate in MPAL was much lower than that in ALL after induction therapy (22.2 vs 81.8 %, $p < 0.0001$) (Table 4 and Fig. 1). Three cases of ALL in HR didn't get remission and blast cells increased during consolidation therapy and gave up because of adverse outcome and economic burden. Five cases of MPAL relapsed within 18 months after initiating treatment. Among six cases of relapse, four cases showed an isolated bone marrow relapse, one case combined marrow and one case central nervous system (Table 3). On the contrary, only 10 cases of ALL with IR relapsed during maintenance treatment or after maintenance treatment which was regarded as intermediate relapse or late relapse [10]. Among 84 cases of ALL with HR, 19 cases showed intermediate and late relapse. The cohort of ALL with IR and HR had a much lower relapse than that of MPAL (16.3 vs 85.7 %, $p = 0.0002$) (Table 4; Fig. 1). Followed until Aug. 2012, only one patient with IR is still alive, others died of relapse or primary resistance to chemotherapy. Overall survival (OS) for this cohort was much lower than

Table 1 Immunophenotypic findings in 10 cases of pediatric MPAL

Case type/number	B-lineage markers	T-lineage makers	Myeloid markers+	MPO (%)
B + My				
1	CD19,cyCD79a,CD20,CD10		CD13	26.3
2	CD19,cyCD79a,CD10		CD13	10
3	CD19,cyCD79a,CD20			77.5
T + My				
4	cyCD79a*	cyCD3,CD7	CD33	23.3
5	cyCD79a*	cyCD3,CD2,CD7	CD13	9.4
B + T				
6	CD19,cyCD79a,CD10	cyCD3,CD2,CD10,CD7	CD13	4
7	cyCD79a,cyCD22*	cyCD3,CD7		2
8	cyCD79a,cyCD22*	cyCD3,CD2,CD7		3.1
9	CD19,cyCD79a,CD20,CD10	cyCD3,CD10,CD7		2
B + T + My				
10	cyCD79a,cyCD22*	cyCD3,CD7	CD33	6.5

* The express of CD19 was weak, which percentage was less than 20 %

Table 2 Morphologic and Karyotypic findings in 10 cases of MPAL

Case type/number	Morphology	Karyotype	Fusion gene	Blast (%) in BM
B + My				
1	L2	46,XY	BCR/ABL HOX11	56
2	L2	47,XY; + mar,t(9;22)[q34;q11]/46,XY	BCR/ABL HOX11	92
3	L3	47,XY, + 7,i(7)(q10),15q + mar	–	69
T + My				
4	L2	48,XX, + X, + 8[17]/46,XX [3]	–	42
5	L2	85–87,XY [13] /46,XY [2]	dupMLL	64
B + T				
6	L2	46, XY	–	43
7	L2	NA*	NA*	87
8	L2	45, X, –Y, del(9)(p11)[18]/46,XY [2]	–	91
9	L2	46, XX, 14q+	–	75
B + T + My				
10	L2	46, XX	–	87

Unclassified not available for AML or ALL

NA not available

that of ALL cohort (9.5 vs 28.1 months, $p < 0.0001$) (Table 4, Fig. 2).

Discussion

MPAL is a rare disease entity that should be distinguished from ALL with atypical myeloid antigen expression and AML with atypical lymphoid expression [11]. In a study of adults with acute leukemia, biphenotypic disease represented 8 % of cases and conferred a poor prognosis, with a

4-year survival rate of only 8 % [2]. This result was supported by Weir EG et al. [12]. In their report of 16 patients with outcome data, only six achieved complete remission and only two remained disease free 2.5 and 4.5 years after chemotherapy or stem cell transplantation. On the contrary, Killick S et al. [4] described 20 patients with biphenotypic leukemia (8 children and 12 adults) among nearly 700 patients treated at their center. CR was achieved in 70 % of patients, furthermore all 8 children with this type of leukemia achieved CR (5 after ALL induction, 2 after AML induction, and 1 after a switch to AML induction after a

Table 3 Clinical features and response to therapy in 10 children with MPAL

Case/type	Age (year)	Sex	WBC $\times 10^9/L$	Response 1	Response 2	Response 3	Relapse site	Risk	OS (month)
B + My									
1	5.5	M	29.1	PR	CR	CR	BM	HR	9.5
2	7.8	M	68.8	NR	NR	NR	GU	HR	7.4
3	6.2	M	23.7	NR	PR	CR	CNS	HR	6.3
T + My									
4	7.1	F	6.7	NR	CR	CR	BM	HR	13.3
5	13.3	M	88.4	PR	CR	CR	BM,CNS	HR	11.6
B + T									
6	16.1	M	15.9	NR	NR	NR	GU	HR	4.5
7	2.1	M	412	NT	–	–		HR	1.5
8	8.2	M	140	CR	CR	CR	Alive	IR	49.2
9	4.5	F	40.6	CR	CR	CR	BM	IR	12.8
B + T + My									
10	7.5	F	67.7	NR	CR	CR	BM	HR	8.5

WBC indicates white blood cell count, Response 1: after induction therapy; Response 2: after first CAM; Response 3: after second CAM; OS overall survival after diagnosis, CR complete remission (blast $\leq 5\%$), PR partial remission (blast $> 5\%$, $\leq 25\%$), ALL acute lymphoblastic leukemia, NR no response (blast $\geq 25\%$), NT no treatment, GU give up therapy, BM bone marrow, CNS central nervous system, IR intermediate risk, HR high risk

Table 4 Clinical outcomes in patient subgroups of ALL and MPAL

Outcome	Type		<i>p</i>
	ALL	MPAL	
CR (no. of patients)	148 (n = 181)	2 (n = 9)	<0.0001
Rate (%)	81.8	22.2	
Relapse (no. of patients)	29 (n = 178)	6 (n = 7)	0.0002
Rate (%)	16.3	85.7	
OS	ALL (n = 181)	MPAL (n = 10)	<0.0001
Median (months)	28.1	9.5	
95 % CI (%)	26.6–30.6	3.2–24.2	

Three cases of ALL and 2 cases of MPAL didn't get remission before consolidation therapy and gave up who were excluded from relapse and RFS study

CR complete remission, OS overall survival

Fig. 1 Therapeutic response and relapse between ALL and MPAL. **a** (Left) The CR rate for ALL with IR and HR was 81.8 %, which was statistic higher than that of MPAL(22.2 %) ($p < 0.0001$). **b** (Right) Relapse rate in MPAL was 85.7 % which was much higher than that of ALL cohort (16.3 %), $p = 0.0002$

Fig. 2 Overall survival(OS) of MPAL and ALL The overall survival of ALL was 82.9 %, which was much higher than that of MPAL (10 %), $p < 0.0001$

poor response to ALL induction). The authors suggested that the prognosis associated with biphenotypic leukemia in children may be same as that typically reported for childhood acute leukemia. However, the outcome of MPAL in our study was even poorer with 10 % long-term survival. The main reason for it is due to the stubborn therapy on CCLG-2008 protocol. CCLG-2008 protocol was first implemented as a clinical trial when our nine cases of MPAL were enrolled in this study. According to the principle of CCLG-2008 protocol, all patients who were enrolled in were permitted to adapt the therapy on risk. HR patients who didn't respond well to the remission therapy were permitted to continue the therapy if their parents didn't like to change after well communication. As a result, nine cases of MPAL were treated following risk-adapted treatment of the protocol and no change in their treatment even though they didn't get remission after induction therapy with ALL. When we retrospectively analyzed these cases, we do find that CCLG-2008 protocol didn't work well for MPAL.

MPAL has specific biologic characters and needs novel treatment for the goal of long term survival. Dr Rubnitz reported their experience in treating MPAL in St. Jude Children's Research Hospital. They proposed that treatment for MPAL begins with one course of AML-type induction therapy, with a provision for a switch to lymphoid-type induction therapy with a glucocorticoid, vincristine, and L-asparaginase if the patient responds poorly [13], and vice versa [14]. Matutes E et al. [14] reported their study in 100 patients of MPAL. Response to treatment and outcome demonstrated that ALL treatment induced a

response in 85 %, AML therapy in 41 %; three of 5 patients responded to the combination therapy. Forty (58 %) patients died, 33 of resistant disease. Overall median survival was 18 months and 37 % of patients were alive at 5 years. Age, Ph(+), and AML therapy were predictors for poor outcome. However, our cases who were treated with ALL therapy didn't response well which confirmed to be poor-risk disease. From our failed experience and other successful reports, we can conclude that the treatment of MPAL needs personalized and switched therapy presently and novel therapy in future.

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