End of induction minimal residual disease alone is not a useful determinant for risk stratified therapy in pediatric T-cell acute lymphoblastic leukemia

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Abstract

The role of end of induction minimal residual disease (MRD) as determined by flow cytometry for treatment assignment in pediatric T-cell acute lymphoblastic leukemia (T-ALL) is not well defined. We studied 33 children with newly diagnosed T-ALL. Thirty-two of 33 patients remain in continuous complete remission at a median of four years. Nineteen patients were MRD positive at the end of induction and all remain in remission with augmented Berlin Frankfurt Münster-based therapy. One patient underwent hematopoietic stem cell transplant for rising MRD. Persistent end of induction MRD alone is not an indication to alter therapy in pediatric T-ALL.

Keywords

Pediatric; T-ALL; Minimal residual disease

Introduction

The role of end of induction minimal residual disease (MRD), detected by flow cytometry, in treatment allocation in pediatric T-cell acute lymphoblastic leukemia (T-ALL) is not well defined. In this study we investigated the prognostic value of MRD, as detected by flow cytometry, in T-ALL. Although we detected MRD at the end of induction in over half the patients, there were no relapses among the MRD positive patients who were treated with various regimens based on the Children's Oncology Group (COG) augmented Berlin-Frankfurt-Münster backbone without hematopoietic stem cell transplantation (HSCT). Our findings suggest that (1) despite slow clearance of blasts, patients who have MRD at the end of induction may have good outcomes with commonly used higher risk ALL regimens; (2) end of induction MRD alone is not predictive of clinical outcomes, and is not an indication

for the modification of treatment; and (3) studies focused on MRD clearance at later time points may be needed to define optimal treatment allocation strategies in T-ALL.

MRD is commonly measured by flow cytometry in North America or polymerase chain reaction (PCR) for immunoglobulin or T-cell receptor gene rearrangements in Europe. PCR is more sensitive but requires leukemia specific re-arrangements to be characterized at diagnosis, may fail to detect new rearrangements related to clonal evolution [1], does not discriminate dead from live cells, and has limited availability. Flow cytometry is a readily available sensitive method for the detection of MRD, which can discriminate between live and dead cells after treatment, and uses standardized predetermined antibody panels. While there are numerous studies showing the strong prognostic value of end of induction MRD in B-precursor (BP)-ALL [1–3], there are few studies of MRD in T-ALL, a less common disease that accounts for under a fifth of childhood leukemias [4]. Thus, while end of induction MRD detected by flow cytometry is an established determinant for treatment allocation in BP-ALL [3], its role in the management of T-ALL is much less defined. Differences in sensitivity and assay characteristics between PCR and flow cytometry, and the increasingly prevalent clinical use of flow cytometry for MRD assessment make it imperative to specifically determine the prognostic value of MRD detected by flow cytometry, in T-ALL.

Methods

All children aged one to 21 years with newly diagnosed T-ALL that were treated at Children's Hospital Los Angeles (CHLA) between January 2006 and December 2012 were included in this historic cohort analysis, which was approved by the CHLA institutional review board. As per physician discretion, patients were treated according to CCG-1961, AALL-0232, or AALL-0434 COG protocols. Therapy consisted of induction, augmented consolidation [5], interim maintenance (high dose [5 gram/meter2] or escalating dose [Capizzi] intravenous methotrexate [5]), one delayed intensification, and maintenance phases. Twenty-one patients received cranial irradiation. One patient underwent HSCT. Bone marrow MRD was analyzed in the CHLA clinical lab by flow cytometry to detect a cluster of events with an aberrant antigen pattern that either resembled that of leukemic cells at diagnosis or was inconsistent with normal hematopoiesis. MRD was defined as 0.01% residual leukemia cells.

Results

Our cohort included 33 patients (Table I). MRD was evaluated at the end of induction in 32 patients. Nineteen patients (59%) were MRD positive at the end of induction. Assessment during consolidation revealed no MRD in the one patient who was not evaluated at the end of induction. We found no associations between age, sex, WBC count at diagnosis, ethnicity, overweight/ obese weight status, cytogenetics, immunephenotype, or type of steroid used in induction, and the risk for end of induction MRD (Table I). MRD was persistently positive in 6 of 11 patients tested at the end of consolidation, and 2 of 4 patients tested at the end of interim maintenance (Table II). The MRD level declined in 10 of 11 patients. All 19 MRD positive patients and 13 of 14 MRD negative patients were in

continuous complete remission at a median follow up of four years (range 1.3–7.1 years, 31 patients followed >two years). One patient underwent HSCT for rising MRD 5.4 months after diagnosis. One patient who was MRD negative at the end of induction had a bone marrow relapse 18 months after diagnosis (alive with refractory disease at last follow up). There were no significant differences in treatment variables (dexamethasone, high dose methotrexate, cranial irradiation) between MRD positive and negative patients.

Discussion

Although more than 50% of patients were MRD positive at the end of induction, there were no relapses among MRD positive patients during a follow up that exceeded the time frame (two years from diagnosis) when most events in T-ALL occur [9]. Our results suggest that persistent end of induction MRD is not strongly predictive of relapse in T-ALL.

The end of induction MRD rate in our cohort was lower than that seen in a PCR based T-ALL study [10]. The detection of non-viable leukemic cells by PCR, as well as methodological and sensitivity differences can result in quantitative as well as qualitative discordances between PCR and flow cytometry, especially at the end of induction time point [11]. This warrants the need for evidence from flow cytometry based MRD studies in order to guide specific therapy decisions in patients assessed by flow cytometry.

Although small subgroup numbers precluded a detailed analysis, unlike in BP-ALL, overweight/ obese weight status was not associated with the risk for end of induction MRD in our cohort [12]. The end of induction MRD rate was higher than that seen in high risk BP-ALL [13], suggesting that the clearance of leukemic cells in response to chemotherapy is slower in T-ALL. Nonetheless, MRD positive patients in our cohort had an excellent outcome, suggesting that the slower clearance of blasts in T-ALL may not have an impact on clinical outcome. Of note, a majority of our patients were Hispanic, an ethnic group that has been described to have poorer outcomes in ALL [14]. The persistent MRD during serial assessments in our patients is consistent with studies showing a higher rate of residual disease [10,15] in T-ALL when compared with BP-ALL.

Preliminary results suggested excellent outcomes in a recent COG study in which, end of induction MRD   0.1% was one variable that was used to stratify risk and test intensification of therapy with a novel T-cell specific agent in a randomized fashion for patients with National Cancer Institute standard risk disease [16]. On the other hand our results suggest that a significant proportion of end of induction MRD positive T-ALL patients may have good outcomes with various commonly used front line high risk ALL regimens, and further modification of treatment based on end of induction MRD alone may not be necessary. Our results are consistent with those seen in a European study where 63% of patients had PCR detectable MRD at the end of induction and undetectable or low levels of MRD at the end of consolidation but still had good outcomes (event free survival of 80.6%) [10].

Our study provides evidence in the context of flow cytometry that end of induction as the sole time point for MRD assessment may not represent an optimal strategy for guiding treatment allocation in T-ALL. Also, the lack of relapses in MRD positive patients despite

persistent MRD at the end of consolidation in at least a third of them raises the need to define the role of MRD clearance kinetics in T-ALL risk stratification. Given the low incidence of T-ALL [4], and the treatment dependent predictive value of prognostic factors [17], multicenter studies of MRD assessment by flow cytometry at multiple time points, in the setting of different treatment protocols, are needed to define risk stratification strategies.

Acknowledgements

Abbreviation key

MRD	Minimal residual disease
T-ALL	T-cell acute lymphoblastic leukemia
HSCT	Hematopoietic stem cell transplantation
BP-ALL	B precursor-acute lymphoblastic leukemia
PCR	Polymerase chain reaction
COG	Children's Oncology Group

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Table I

Patient, disease, and treatment characteristics stratified by end of induction MRD

Variables Total		All patients N=33	MRD positive N=19	MRD negative N=13	Association between variable and risk for MRD
Age (years)[a]	median (range)	10 (1–20)	11 (2–17)	10 (1–20)	P>0.05[b]
	10 years	19	11	8	
	<10 years	14	8	5	
Gender					
	Male	23	13	10	P>0.05[b]
	Female	10	6	3	
Ethnicity					
	Hispanic	18	13	5	P>0.05[b]
	Non-Hispanic	15	6	8	
Weight status[a]					
	Overweight/ obese	12	7	5	P>0.05[b]
	Lean	18	12	6	
	NA	3		2	
WBC[a]	median (range)	77.15 (0.42–793)	79 (0.42–793)	75.3 (2.94–394)	P>0.05[b]
	100	15	9	6	
	<100	17	10	7	

Variables	All patients	MRD positive	MRD negative	Association between variable and risk for MRD
Total	N=33	N=19	N=13	
50	20	13	7	P>0.05[b]
<50	12	6	6	
NA	1			
Cytogenetics				
Normal	20	11	9	P>0.05[b]
Abnormal	13	8	4	
Immunephenotype				
ETP/near-ETP	6	4	2	P>0.05[b]
Non-ETP	26	15	11	
Unknown	1			
CNS disease[c]				
No	32	18	13	
Yes	1	1	0	
NCI/Rome risk category[d]				
Standard	4	3	1	
High	28	16	12	
NA	1			
Induction steroid				
Dexamethasone	18	11	6	P>0.05[b]

Variables Total	All patients N=33	MRD positive N=19	MRD negative N=13	Association between variable and risk for MRD
Prednisone	15	8	7	
Methotrexate dosing[e]				
High dose	14	9	4	
Capizzi	19	10	9	
Cranial Irradiation[e]				
Yes	21	13	8	
No	12	6	5	
HSCT				
Yes	1	1	0	
No	32	18	13	

MRD - bone marrow minimal residual disease at the end of induction. WBC - white blood cell count at diagnosis ($\times 10^3/\mu l$). HSCT - hematopoietic stem cell transplantation. NA - not available. ETP - early thymic progenitor. Weight status was defined according to Center for Disease Control body mass index criteria [6]. ETP and near-ETP immunephenotypes were defined based on published flow cytometry criteria [4,7].

[a] p>0.05 when age, WBC count, or body mass index percentile were analyzed as continuous variables for association with MRD (positive or negative) using univariate logistic regression.

[b] Fisher's Exact test for categorical variables.

[c] CNS disease was defined as 5 white blood cells/μl, and presence of leukemic cells in cerebrospinal fluid.

[d] NCI(National Cancer Institute)/ Rome risk criteria [8].

[e] p>0.05 when the z test of proportions was used to analyze for a difference in proportions for high dose methotrexate or cranial irradiation between MRD positive and negative patients.

Table II

Minimal residual disease (MRD) evaluation results

	Time points			
	End of induction N=32	**Mid-consolidation N=4**	**End of consolidation N=11**	**End of interim maintenance N=4**
MRD				
Negative	13	1	5	2
0·01 to <0·1%	4	1	3	1
0·1 to <1%	10	2	2	1
1%	5		1	