Absence of Biallelic TCRγ Deletion Predicts Induction Failure and Poorer Outcomes in Childhood T-cell Acute Lymphoblastic Leukemia

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Complete List of Authors:	Yang, Yung-Li; National Taiwan University Hospital, College of Medicine, National Taiwan University, Pediatrics Hsiao, Chih-Cheng; Chang Gung Memorial Hospital-Kaohsiung Medical Center, College of Medicine, Chang Gung University, Pediatrics Chen, Hsuan-Yu; Institute of Statistical Science Academia Sinica Lin, Kai-Hsin; national taiwan university hospital, pediatrics Jou, Shiann-Tarng; National Taiwan University Hospital, College of Medicine, National Taiwan University, Pediatrics Chen, Jiann-Shiuh; National Cheng Kung University Hospital, Pediatrics Chang, Te-Kau; Veterans General Hospital–Taichung, Pediatrics Sheen, Jiunn-Ming; Chang Gung Memorial Hospital-Kaohsiung Medical Center, College of Medicine, Chang Gung University, Pediatrics Yu, Sung-Liang; College of Medicine, National Taiwan University, Clinical Laboratory Sciences and Medical Biotechnology Lu, Meng-Yao; National Taiwan University Hospital, College of Medicine, National Taiwan University, Pediatrics Cheng, Chao-Neng; National Cheng Kung University Hospital, Department of Pediatric Hematology-Oncology Wu, Kang-Hsi; China Medical University Hospital, Pediatrics Wang, Shih-Chung; Changhua Christian Hospital, Pediatrics Wang, Jiaan-Der; Taichung Veterans General Hospital Chang, Hsiu-Hao; National Taiwan University Hospital, Pediatrics Lin, Shu-Rung; College of Science, Chung-Yuan Christian University, Bioscience Technology Lin, Shu-Wha; College of Medicine, National Taiwan University, Department of Clinical Laboratory Sciences and Medical Biotechnology Taipei, Taiwan Lin, Dong-Tsamn; College of medicine,National Taiwan university, pediatrics and laboratory medicine
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Absence of Biallelic *TCRγ* Deletion Predicts Induction Failure and Poorer Outcomes in Childhood T-cell Acute Lymphoblastic Leukemia

Yung-Li Yang, MD,[1,2,3], Chih-Cheng Hsiao, MD,[4], Hsuan-Yu Chen, PhD,[5], Kai-Hsin Lin, MD,[2], Shiann-Tarng Jou, MD,[2], Jiann-Shiuh Chen, MD,[6], Te-Kau Chang, MD,[7], Jiunn-Ming Sheen, MD,[4], Sung-Liang Yu, PhD,[8], Meng-Yao Lu, MD,[2], Chao-Neng Cheng, MD,[6], Kang-Hsi Wu, MD,[9,10], Shih-Chung Wang, MD,[11], Jiaan-Der Wang, MD,[7], Hsiu-Hao Chang, MD,[2], Shu-Rung Lin, PhD,[12], Shu-Wha Lin, PhD,[8], and Dong-Tsamn Lin, MD,[1,2]

Corresponding authors:
Dong-Tsamn Lin, MD.
No.7, Chung-Shan S. Road, Taipei, Taiwan
Department of Laboratory Medicine and Pediatrics
National Taiwan University Hospital, Taipei, Taiwan
Tel: 886-2-2312-3456 ext. 65399;
Fax: 886-2-23224263
E-mail: dtlin@ntuh.gov.tw

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[1]Department of Laboratory Medicine, [2]Department of Pediatrics, National Taiwan University Hospital, [8]Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, Taipei, Taiwan
[3]Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan
[4]Department of Pediatrics, Chang Gung Memorial Hospital-Kaohsiung Medical Center, College of Medicine, Chang Gung University, Kaohsiung, Taiwan
[5]Institute of Statistical Science Academia Sinica, Taipei, Taiwan, Republic of China
[6]Department of Pediatrics, National Cheng Kung University Hospital, Tainan, Taiwan
[7]Department of Pediatrics, Veterans General Hospital–Taichung, Taichung, Taiwan
[9]Department of Pediatrics, Children's Medical Cancer, [10]School Chinese and Medicine, China Medical University Hospital, Taichung, Taiwan
[11]Department of Pediatrics, Changhua Christian Hospital, Changhua, Taiwan
[12] Department of Bioscience Technology, College of Science, Chung-Yuan Christian University, Taoyuan, Taiwan.

ABSTRACT

Background. The absence of biallelic *TCR*γ deletion (ABD) is a characteristic of early thymocyte precursors before V(D)J recombination. The ABD was reported to predict early treatment failure in T-cell acute lymphoblastic leukemia (ALL). This study aimed to investigate its prognostic value in Taiwanese patients with T-cell ALL.

Procedure. Forty-five children with T-cell ALL were enrolled from six medical centers in Taiwan. Quantitative DNA polymerase chain reaction (Q-PCR) was performed to check the status of *TCR*γ deletion. The threshold for homozygous deletions by Q-PCR was defined as a fold-change less than 0.35.

Results. ABD was found in 20 patients (20/45) who had higher incidences of induction failure than those without ABD (P = 0.03; hazard ratio [HR] = 8.13; 95% confidence interval [95% CI] = 1.23-53.77) after multivariate regression analysis. Patents with ABD also had inferior EFS and OS (P = 0.071 and 0.0196, respectively). Multivariate Cox analysis indicated that the association between ABD and overall survival was independent of age and leukocyte count on presentation (P = 0.036; HR = 4.25; 95% CI = 1.10-16.42).

Conclusions. The absence of *TCR*γ deletion is a predictor of a poor response to induction chemotherapy for pediatric patients with T-cell ALL in Taiwan. Providing patients with T-cell ALL and ABD with alternative regimens may be worthwhile to test in future clinical trials.

INTRODUCTION

Children with T-cell acute lymphoblastic leukemia (ALL) are considered to have intermediate-to-high risk of relapse (1, 2). Although the use of intensive chemotherapy has enabled patients with T-cell ALL to fare as well as patients with B-cell precursor ALL in some studies, the outcomes are significantly worse for the patients with T-cell ALL in most treatment protocols (3-15). Chromosomal alterations, including numbers and translocations, have helped to classify pediatric patients with B-cell precursor ALL and improve treatment outcomes in the past three decades (16). Because prognostic factors for pediatric T-ALL have been unreliable, with only marginal differences in outcomes for sub-groups defined by the cell-marker expression of leukemic lymphoblasts in early studies, pediatric patients with T-cell ALL have been treated uniformly in all major study protocols (17).

Demographic and clinical features, such as age at onset and initial white blood cell counts, are not reliable predictive parameters of the T-cell type of ALL (16, 18). Identifying prognostic markers that stratify patients with T-cell ALL may improve risk-directed therapies and help find novel therapeutic targets for patients who fail initial chemotherapy. Information on the genetic development of T-cell ALL has been obtained from gene expression profiles, some of which is associated with clinical outcomes (19, 20). Some studies have constructed gene expression signatures to predict clinical outcomes for T-cell ALL (21-23). With advances in the molecular typing of leukemia, many new genetic alterations, including mutations and deletions, have been identified. However, their prognostic values have not been widely verified and the results may be controversial (24-34).

Two independent studies identified pediatric patients with T-cell ALL that had very poor initial treatment responses and clinical outcomes (35, 36). Coustan-Smith et

al. found that patients with early T-cell precursor leukemia (ETP-ALL), accounting for around 12% of pediatric T-cell ALL, had very high incidences of treatment failure (35). The prognostic value of ETP-ALL was even more significant than minimal residual disease. Gutierrez et al. identified a subgroup of pediatric patients with T-cell ALL with an absence of *TCR*γ deletion (ABD) who had an increased risk of induction failure and dismal outcomes regardless of salvage treatment (36). Gutierrez et al. also demonstrated biologic overlap between ABD and ETP-ALL by gene expression signature. In addition, regardless of whether patients with T-cell ALL had ABD or ETP-ALL, their clinical course was aggressive and they exhibited early induction failure and inferior overall survival rates (36).

This study used the quantitative DNA polymerase chain reaction (Q-PCR) designed by Gutierrez et al. to check the status of *TCR*γ deletions (36), and analyzed the prognostic value in patients with T-cell ALL who received the Taiwan Pediatric Oncology Group (TPOG) treatment protocols in Taiwan.

PATIENTS AND METHODS

Patients and protocols

After receiving Institutional Review Board approval, diagnostic samples were collected. Written informed consent was obtained from the patients or their families. The diagnosis of T-cell ALL was based on bone marrow aspiration and immuno-phenotyping with monoclonal antibodies directed to T-lineage-associated antigens (7). Between January 1997 and August 2010, 45 pediatric patients with T-cell ALL were enrolled from six medical centers in Taiwan. Seven had paired relapse samples. All were treated according to the Taiwan Pediatric Oncology Group (TPOG) protocols. Patients received TPOG-ALL-97 VHR for chemotherapy if they were diagnosed

between January 1997 to December 2001, and TPOG-ALL-2002 VHR if they were diagnosed between January 2002 and August 2010 (7). The TPOG-ALL-2002 VHR protocol differed from TPOG-ALL-97 VHR (activated in January 1997) in prednisolone (decreased from 60 mg/m^2/day to 40 mg/m^2/day) and dexamethasone (decreased from 8 mg/m^2/day to 6 mg/m^2/day) (7).

Response to induction chemotherapy was assessed by examining the bone marrow, peripheral blood, and central nervous system (CNS) for evidence of persistent leukemia four weeks after starting the multi-agent induction chemotherapy. Complete remission was defined as < 5% lymphoblasts in the bone marrow with no evidence of peripheral blood, CNS, or extra-medullary leukemia in the setting of restored normal hematopoiesis and hematologic recovery. Patients who failed to achieve complete remission were classified as having induction failure.

Immunophenotypes

Immunophenotyping was performed at the clinical diagnostic laboratory used by each medical center. The definition of the early T-cell precursor immunophenotype was as described by Coustan-Smith and colleagues: absent (< 5%) CD1a and CD8, weak (<75%) CD5, and an expression of 25% or more of one or more of the following: CD117, CD34, HLA-DR, CD13, CD33, CD11b, or CD65 (35).

DNA extractions

Mononuclear cells were purified from diagnostic bone marrow or peripheral blood specimens by Ficoll-Paque centrifugation according to the manufacturer's instructions (GE Healthcare, Piscataway, NJ) prior to cryopreservation. All specimens analyzed consisted of >90% lymphoblasts. DNA samples were prepared using proteinase-K digestion, saturated sodium chloride deproteinization, and isopropanol/ethanol precipitation (37).

Quantitative DNA PCR for the absence of biallelic TCRγ deletion

Quantitative DNA PCR (Q-PCR) for *TCR*γ re-arrangements was performed using the method designed by Gutierrez et al. (36). The quantitative DNA-PCR primer sequences were *TCR*G-VJ forward, CATCCTCACTTTCCTGCTTCTTC and *TCR*G-VJ reverse, CCAAGGTGAATCCCTACATGCT; *ANLN* forward, AAATTCTGCCCTTTGCTTGTTT and *ANLN* reverse, GAAAGCAACCACAGAGAATATGTAAGTAA. Quantitative DNA PCR was carried out using genomic DNA 50 ng, 1 micro-molar primer 3 mcl, and Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) 7.5 mcl in a total volume of 15 mcl per reaction, using an Applied Biosystems 7500 Fast Real Time PCR System instrument.

The Q-PCR reaction conditions were as follows: initial denaturation step, 94°C x 10 min; 40 PCR cycles at 94°C x 10 sec, then at 60°C x 60 sec. All PCR reactions were performed in triplicate. Results of the *TCR*γ-VJ quantitative PCRs were reported as fold-change compared to the *ANLN* Q-PCR. The threshold for homozygous deletions by Q-PCR was defined as a fold-change less than 0.35 (corresponding to less than 35% of the normal copy number) as determined by Gutierrez et al. (36).

Statistical analysis

Fisher's exact test for categorical data was used to compare baseline clinical variables across groups. EFS was defined as the time from diagnosis to the date of the last follow-up in complete remission or the first event (i.e., induction failure, relapse, secondary neoplasm, or death from any cause). Failure to achieve remission due to non-response was considered an event at time zero. Survival was defined as the time from diagnosis to death or the last follow-up. Patients lost to follow-up were censored at the last contact. The Kaplan-Meier method was used to estimate survival rates, with

the differences compared using a two-sided log-rank test.

Cox proportional hazard models were constructed for EFS and OS, and used for univariate and multivariate analyses. Covariates included in the full model of OS and EFS were ABD, WBC ($<10 \times 10^9$/L, between 10×10^9/L to 100×10^9/L, and $\geq 10 \times 10^9$/L), and age (<10 years *vs.* >10 years). Step-wise backward selection was performed. All analyses were performed using the SAS statistical software package, version 9.0 (SAS Institute, Cary, NC).

RESULTS

Association of ABD with immunophenotypes

Immunophenotypes, available for 43 of the 45 patients in our series, are listed in Supplemental Table I. Recent work by Coustan-Smith et al. identified that ETP-ALL could be defined by immunophenotype, and that it could predict treatment failure in pediatric patients with T-cell ALL (35). Gutierrez et al. demonstrated an overlap between ABD and early T-cell precursor ALL by ETP gene expression signatures (36). In contrast with the overlap of ABD and ETP-ALL by ETP gene expression, only one patient in Gutierrez et al.'s series met the criteria of ETP-ALL by immunophenotyping (36). Although CD1a was not included in the diagnostic panels in this study, we attempted to identify the possible ETP-ALL cases by weak CD5 expression and other markers. However, as in Gutierrez et al.'s series (36), most of our patients exhibited high CD5 expression in diagnostic immunophenotyping. In this series, nine patients suffered from induction failure and eight of them had ABD. Only one of these patients had a CD5 expression below 75% and met the criteria of ETP-ALL, with the exception of the unavailability of CD8 expression (case number 41, Supplemental Table I). With available stored cells for immunophenotyping of this

patient, we demonstrated this case was ETP-ALL with the inclusion of diagnostic markers including CD1a and CD8 (Fig. 1a). In addition to the high expression of CD5, the lack of CD8 expression, but not the absence of CD8 expression (> 5%) made it difficult for us to distinguish the patients with ABD and induction failure as ETP-ALL by prior available immunophenotypes. We also re-ran the diagnostics on stored cells for case number 16. This patient met the criteria of ETP-ALL with the exception of CD5 expression (Fig. 1b). We suspect that our laboratory did not accurately distinguish between low (but not absent) CD5 expression characteristics of ETP lymphoblasts and the higher CD5 expression present in the majority of T-ALL patient samples.

ABD was associated with induction failure and poorer overall survival

The clinical characteristics of patients at the time of diagnosis are presented in Table I. Twenty patients had ABD. They had higher incidences of induction failure than those without ABD (P = 0.019, by Fisher's exact test; hazard ratio [HR] = 7.67; 95% confidence interval (95% CI) = 1.4-41.94). Patients with ABD had inferior five- and ten-year OS than those without ABD (52.5±11.7% vs. 86.23±7.4% and 45.94±11.9% vs. 86.23±7.4%, respectively) (P=0.0196, Fig. 2), and inferior five- and ten-year EFS (42.66±11.5% *vs*. 72.79±9.7% and 42.66±11.5% *vs*. 72.79±9.7%, respectively) (P=0.07, Fig. 3). The prognostic impact for EFS was marginal.

ABD was an independent prognostic factor for induction failure and overall survival

Multivariate Cox regression analyses were used to examine the correlation between EFS or OS and ABD status, age, and presenting white blood cell (WBC) count (Table I). ABD was an independent prognostic factor for induction failure (HR = 8.13; 95% CI = 1.23-53.77; P=0.03) and OS (HR = 4.25; 95% CI = 1.10-16.42;

P=0.036) (Table II). However, the multivariate analysis indicated that ABD for EFS was not statistically significant (*P*=0.129).

ABD in relapsed T-cell ALL

Seven paired samples relapsed after induction chemotherapy. Five patients had ABD in the initial diagnostic samples and in the relapsed clones. However, two patients with biallelic *TCRγ* deletions on diagnosis had relapsed clones characterized by ABD. Some of the matched newly-diagnosed and relapsed samples by Mullighan and Ferrando laboratories also exhibited a discrepancy with this marker between diagnosis and relapse (36).

DISCUSSION

In this study, pediatric patients with T-cell ALL with ABD appear more likely to experience induction failure and inferior overall survival, as also seen in the series of Gutierrez et al. (36). Twenty percent patients without ABD achieved remission but relapsed in a short time (3-6 months) after induction chemotherapy. Therefore, multivariate analysis failed to show that ABD is an independent prognostic factor for EFS (*P*=0.129). The higher induction failure rate for patients with ABD made it necessary for us to provide patients with T-cell ALL and ABD with alternative treatment strategies to improve the treatment responses.

Patients with T-cell ALL have a higher incidence of induction failure and CNS relapse (15, 38). Compared with precursor B-cell ALL, the prognostic markers for T-cell ALL are relatively inadequate for treatment planning. In various Berlin-Frankfurt-Műnster (BFM)-like treatment protocols, patient stratification is based primarily on patient response to glucocorticoid-based prophase therapy and minimal residual disease analysis (9, 39). Two reports that identified high-risk T-cell ALL

with primary failure may provide an avenue for molecular risk-directed therapy for T-cell ALL (35, 36). Coustan-Smith et al. identified a sub-group of patients with early T-cell precursor leukemia. This group had several unique clinical features, such as gene expression profiles similar to thymic precursors, and unique immuno-phenotypes (i.e., $CD1a^{-}$, $CD8^{-}$, $CD5^{weak}$ with myeloid or stem cell markers) (35). These patients had very poor outcomes, equal to or even worse than those of patients with *BCR-ABL1*. Later, Gutierrez et al. identified a group of patients with ABD through array comparative genomic hybridization (CGH)(36). The clinical features of this group were very similar to the presentations of patients with early precursor T-cell ALL. The patients also had high incidences of induction failure and dismal outcomes even with salvage protocols. Because *TCRγ* rearrangements occur early in normal T-cell development, and deletions of *TCR* loci are significantly less frequent in ETP-ALL, Gutierrez et al. demonstrated a biologic overlap of patients with ABD and ETP-ALL by ETP gene signatures (36). The identification of T-cell ALL with ABD by Q-PCR or ETP-ALL by immunophenotypes as high-risk T-cell ALL may be important in the stratification of risk for pediatric T-cell ALL in future clinical trials.

Gutierrez et al. (36) were not able to correlate the immunophenotypes of ABD with ETP profiles of surface markers as described by Coustan-Smith et al.(35). For immunophenotyping, the authors attributed this discrepancy to the interpretation of the detection of CD5 more than 75 % in all but one of their patients. The authors suspected that the use of a threshold based on the percentage of positive blasts may have failed to accurately distinguish CD5-low ETP blasts from CD5-high patients with T-ALL in their series. As was the case in Gutierrez et al. study (36), most of our patients had high CD5 expression and failed to meet the criteria of ETP-ALL. In our case series, nine patients suffered from induction failure, and eight of them had ABD.

Only one of these patients had a CD5 expression below 75% and met the criteria of ETP-ALL, except for the unavailability of CD8 expression (case number 41, supplemental Table I). With available stored cells for immunophenotyping of this patient, we demonstrated that the case was ETP-ALL with the inclusion of diagnostic markers of CD1a and CD8 (Fig. 1a). However, just as in the series of Gutierrez et al. (36), our laboratory could not distinguish positive CD5-low ETP and CD5 high T-cell ALL for most patients with ABD and induction failure (Fig. 1b). To identify the patients with ETP-ALL correctly by immunophenotypes warrants further investigations in the future.

The reasons for which patients with ABD respond poorly to initial induction chemotherapy remain largely unknown. In normal human T-cell development, most normal human pro-thymocytes have not re-arranged *TCR*γ. *TCR*γ deletions occur in the early phase of T-cell development. The absence of re-arrangement means that this leukemic clone develops in the very early phase of T-cell development. The same is true for precursor B-cell ALL. Although *PAX5* alterations are the most frequent genetic abnormality in precursor B-cell ALL (40), the loss of *PAX5* is not associated with poor outcomes, possibly because of a lack of deregulation in stem cell-associated genes (41). In contrast, deletion or sequence mutation of the *IKZF1* gene, encoding the early lymphoid transcription factor *Ikaros*, increases the risk of treatment failure (42-45). It appears that when leukemias occur in the earlier phases of lymphocyte development, the prognosis is poorer for childhood ALL, irrespective of whether it is B- or T-cell ALL.

This study screened seven paired samples with relapse, all of which had ABD. However, two of them had initial samples without ABD. Similar results have been obtained for progenitor B-cell ALL. *IKZF1* deletions have recently been identified as

a poor prognostic marker for relapse in progenitor B-cell ALL (42). Interestingly, its alterations are also the top three frequently encountered new genetic alterations in relapsed samples. From these studies, the emergence of relapsed clones with poor prognostic markers, regardless of whether in B- or T-cell ALL, is therapeutically challenging because such patients are not classified as high-risk patients on initial diagnostic screenings.

In conclusion, ABD predicted induction failure in pediatric patients with T-cell ALL in this study based in Taiwan. Because this case series was relatively small, the most effective induction chemotherapy for patients with ABD and their immunophenotyping association with ETP-ALL warrants further investigation.

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AUTHORSHIP

The author contributions were as follows: YL Yang was responsible for literature collection, data management and interpretation, and writing the manuscript; SW Lin and SR Lin were responsible for the design of the mutation analysis and interpretation

of the results; SL Yu and HY Chen were responsible for performing the statistical analyses and the interpretation of the statistical findings; JS Chen, KH Lin, ST Jou, CC Hsaio, TK Chang, JD Wang, JM Sheen, CN Cheng, KH Wu, MY Lu, and HH Chang contributed patient samples and clinical data; DT Lin, and SW Lin planned, designed, and coordinated the study over the entire period and wrote the manuscript.

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LEGEND

Figure 1. (a) The phenotypic profile, with absent expression of CD1a and CD8, low expression of CD5, and expression of CD13 met the criteria of ETP-ALL (case number 41). (b) The phenotypic profile of another patient with T-cell ALL with ABD met the criteria of ETP-ALL, except for the expression of CD5 (case number 16). The percentage of blasts was slightly different from the data in supplemental Table I because we re-performed the immunophenotyping of these two stored samples.

Figure 2. Kaplan-Meier analyses of overall survival for patients with T-cell ALL classified by ABD status.

Figure 3. Kaplan-Meier analyses of event-free survival for patients with T-cell ALL classified by ABD status.

TABLE I. Demographic characteristics of pediatric patients with T-cell ALL in this study

	TCRγ deletion	ABD	*P* value[a]
Age	10.78±3.7	10.04±4.8	0.865
Gender			
Male	16 (64.0%)	13 (65.0%)	1.000
Female	9 (36.0%)	7 (35.0%)	
WBC ($\times 10^9$/L)[b]			
<10	1 (4.0%)	4 (20.0%)	0.033
10- <100	14 (56.0%)	14 (70.0%)	
≥100	10 (40.0%)	2 (10.0%)	

[a]Fisher's exact test and Wilcoxon test;[b]WBC= white blood cell

TABLE II. Multivariate Cox regression analysis for induction failure, OS and EFS

Induction failure

Variable	Hazard Ratio	95% Hazard Ratio C.I.		*p* value
ABD	8.13	1.23	53.77	0.030
Gender	1.88	0.29	12.01	0.506
Age>10	1.31	1.00	1.72	0.051
WBC ($\times 10^9$/L)[a]				
<10	1.00			
10- <100	0.18	0.01	2.36	0.191
≥100	0.06	0.002	1.98	0.115

OS

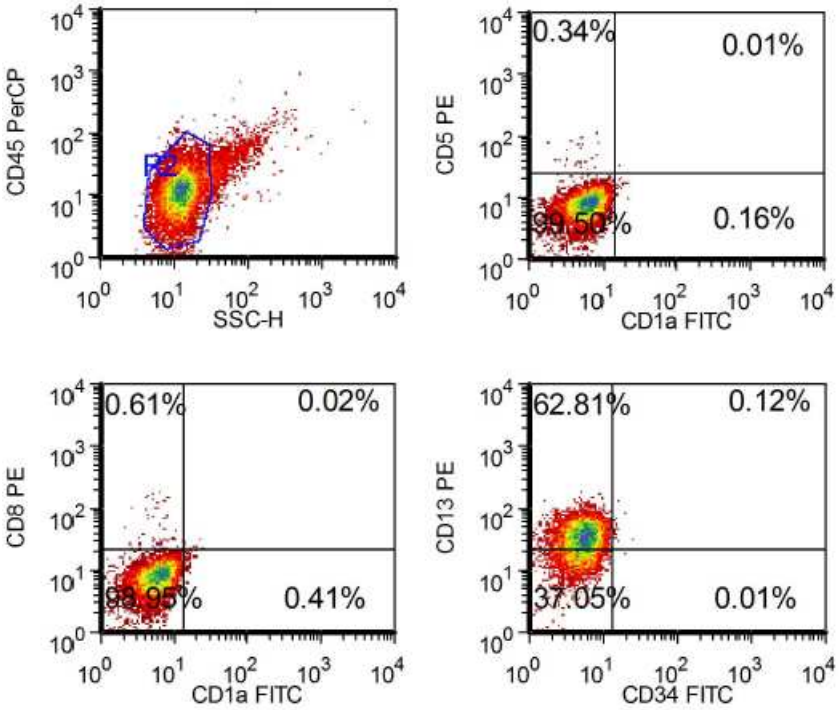
Variable	Hazard Ratio	95% Hazard Ratio C.I.		*p* value
ABD	4.25	1.10	16.42	0.036
Gender	4.87	0.99	24.00	0.052
Age>10	1.15	0.96	1.38	0.129
WBC ($\times 10^9$/L)				
<10	1.00			
10- <100	0.35	0.06	2.26	0.272
≥100	0.23	0.02	2.54	0.229

EFS

Variable	Hazard Ratio	95% Hazard Ratio C.I.		*p* value
ABD	2.25	0.79	6.44	0.129
Gender	1.62	0.55	4.76	0.384
Age>10	1.06	0.94	1.21	0.34
WBC ($\times 10^9$/L)				
<10	1.00			
10- <100	0.86	0.20	3.75	0.837
≥100	0.36	0.05	2.80	0.327

[a]WBC= white blood cell

a


CD45 PerCP
SSC-H
R2
CD5 PE
CD1a FITC
0.34%
0.01%
99.50%
0.16%
CD8 PE
CD1a FITC
0.61%
0.02%
99.95%
0.41%
CD13 PE
CD34 FITC
62.81%
0.12%
37.05%
0.01%

b

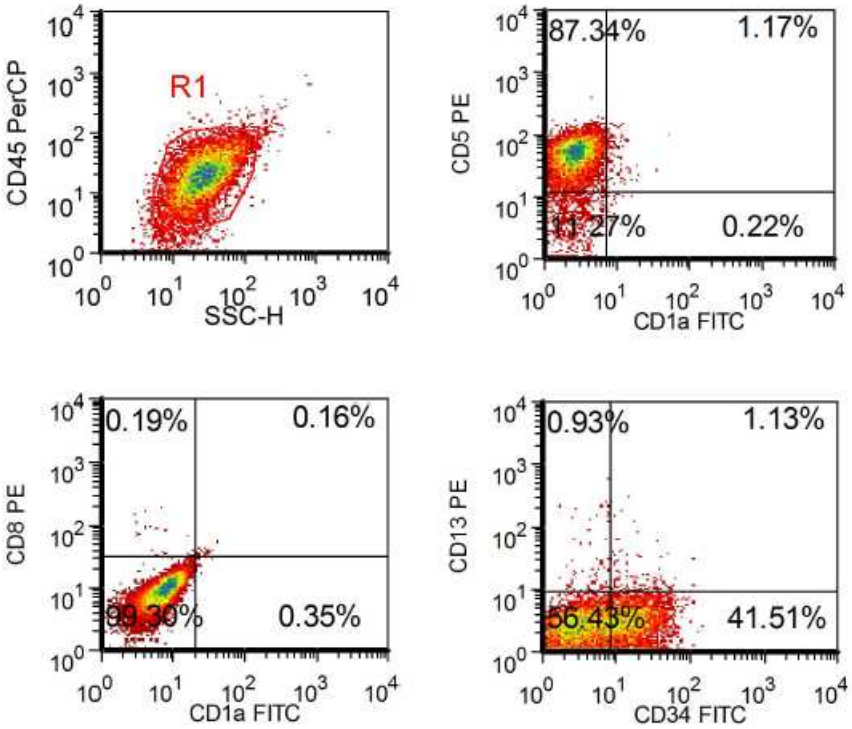

CD45 PerCP
SSC-H
R1
CD5 PE
CD1a FITC
87.34%
1.17%
11.27%
0.22%
CD8 PE
CD1a FITC
0.19%
0.16%
99.30%
0.35%
CD13 PE
CD34 FITC
0.93%
1.13%
56.43%
41.51%

Fig. 1.

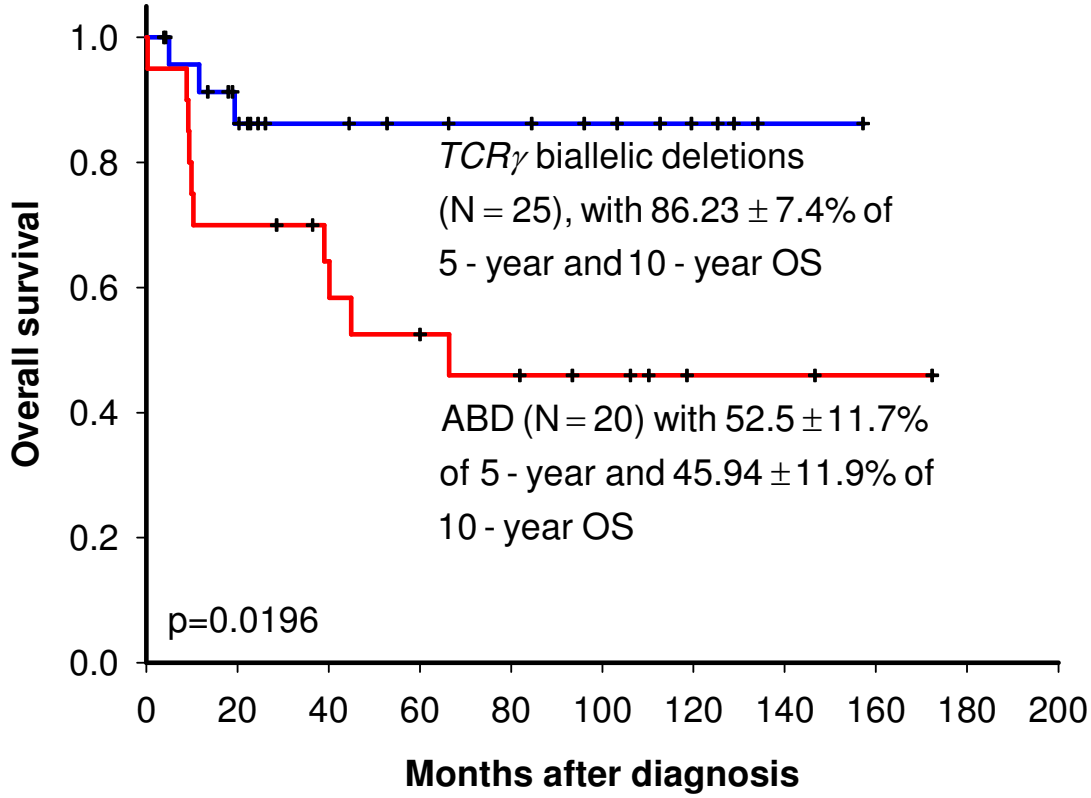


Fig. 2.

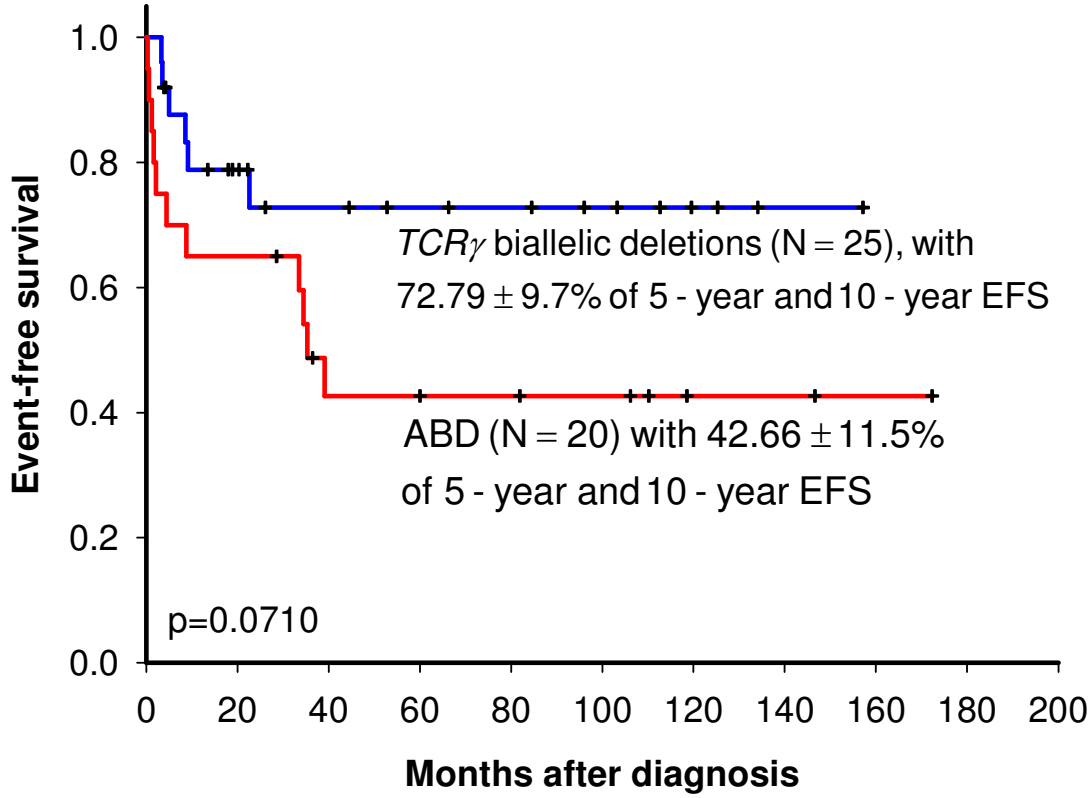


Fig. 3.

Supplemental Table I. The immunophenotypes of study patients.

Case number	ABD	IF[a]	CD19%	CD10%	CD20%	CD7%	CD5%	CD2%	Surface CD3%	CD13%	CD33%	CD15%	HLADR%	CD34%	CD4%	CD8%
1	No	0	0.3	13.8	0.1	99.4	98.8	99.3	1.2	0.9	0.6	4.5	2.8	20.2	92.8	92.1
2	No	0	0	44	0	99	99	99	n/a[b]	n/a	1	n/a	6	62	n/a	n/a
3	No	0	0	24	0	99	30	3	n/a	n/a	96	n/a	0	99	n/a	n/a
4	Yes	0	0	1	1	100	99	56	n/a	n/a	94	n/a	40	63	n/a	n/a
5	No	0	0.9	81.2	n/a	97.9	88.6	98.9	7	9.4	n/a	n/a	3.7	10.2	5	4.4
6	No	0	0.5	23.5	n/a	90.5	89.3	98.2	9.2	2.8	n/a	n/a	1.5	2.7	37.8	59.8
7	No	0	1.3	78.3	n/a	98	94.5	97.8	n/a	6.7	n/a	n/a	8.6	0	94.9	98.2
8	Yes	1	0.5	9	1.4	99.7	99	91.1	1.7	94.6	8.5	2.9	20.6	84	1	0.3
9	Yes	1	18.3	1.4	14.5	77.2	81.5	79.8	16	3.3	1.2	14.1	22.4	3	18.1	33.8
10	No	0	2.3	91.7	1	99.5	99.5	40.5	95.8	0.5	0.1	37	0.5	0.3	48	21.3
11	No	0	0.3	1.1	0.8	94.6	99.2	95.2	79.9	76.4	1.1	1.5	1.6	93.6	0.5	11.9
12	No	0	74	1	4	98	98	99	98	0	1	n/a	81	92	n/a	n/a
13	Yes	0	0.7	36.6	1.2	98.7	96.7	97.8	94.8	43.1	n/a	n/a	1.9	32.3	31.9	83.5
14	No	0	0.9	0	0.7	96	98.5	99	1.6	4.9	0.8	n/a	1.9	3	98	97.7
15	No	0	0.6	0.4	0.8	94.7	99	99.2	81	26	0.5	11.2	1.7	19.1	39	30.8
16	Yes	1	0.4	0	0.3	98.7	98.5	18.2	0.2	3.6	53.9	4.7	5.9	83.2	1.4	n/a
17	No	0	3.1	8	2.2	99.6	99.5	99.6	90.3	40.1	0	0.2	0.8	81	61.9	23.1
18	No	0	0.9	0.8	1.1	99.7	99.8	99.6	4.1	2.2	0.7	5.7	0.9	2.9	76.4	99.5

19	Yes	0	1.4	33.2	n/a	99.6	24.7	98.6	2.6	77.2	17.5	1.3	0.5	61.7	0.5	n/a
20	No	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
21	No	1	0	2	2	99	62	37	n/a	1	1	0	9	23	n/a	n/a
22	No	0	6.9	13.7	1.5	33	51.8	35.9	11.3	0.3	10.6	3.2	1.5	4.6	30.2	30.8
23	No	0	1	0	1	98	96	98	90	1	0	1	0	0	71	51
24	No	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
25	Yes	1	n/a	n/a	n/a	96	97	99	n/a	94	n/a	n/a	n/a	78	n/a	n/a
26	No	0	0	0	0	99	99	99	85	0	0	0	1	0	n/a	n/a
27	No	0	7.4	70.5	8.5	86.2	72.2	84.9	45.8	0.8	23.3	1.5	9.5	9.9	n/a	n/a
28	Yes	0	5.4	5	9.9	82.6	78.4	69.9	13.5	6.7	34	6.4	7.6	23	n/a	n/a
29	Yes	0	8.9	0.7	5.4	69.3	48.5	67.9	42.4	3.3	16.3	n/a	4.8	9.6	n/a	n/a
30	Yes	0	n/a	n/a	n/a	98	97	95	n/a	n/a	n/a	13	12	3	n/a	n/a
31	Yes	0	51	53	5	35	37	41	34	2	0	1	53	4	n/a	n/a
32	Yes	0	5.6	46.9	7.9	93.6	92.2	88.2	13.5	2.7	18.3	5.4	8.1	90.2	4.2	75.8
33	No	0	0.8	20.6	n/a	66.5	64.5	68.4	36.6	2.3	0.5	7	0.9	0.4	61.9	63.7
34	Yes	1	12.8	11.5	1.3	74.9	2.8	3.2	1.2	1.8	76.8	0.9	1.3	0.3	1.4	35.7
35	No	0	2.8	0.6	n/a	96.3	97.4	97.4	8	0.8	0.8	6.4	2.9	0.5	9.8	69.5
36	No	0	1.4	0.7	n/a	99.4	99.6	99.7	92.3	0.6	0.5	1	2.3	0.3	73.7	88.4
37	No	0	1	0.4	n/a	79.1	99.6	99.4	69.4	93.4	3.6	1.6	0.2	95.6	1	63.1
38	No	0	0.7	10	0.3	96.7	6.3	97.2	99.7	98.7	3.9	9.6	2.8	42.2	0.6	2.8
39	Yes	0	2.1	19.4	n/a	97.5	73.2	96.1	4.6	75.3	37	15.8	n/a	84.5	15.9	2.6
40	Yes	1	7.5	0.6	n/a	97.2	93.3	71.5	6.2	11.3	38.5	n/a	n/a	2.4	2.3	40.8

41	Yes	1	10.9	0.2	n/a	98.6	7.8	99.6	0.4	99	98.1	n/a	n/a	0.3	0.1	n/a
42	Yes	0	2.23	0.06	1.92	n/a	62.77	n/a	19.6	n/a	23.93	n/a	5.61	64.06	1.12	4.12
43	Yes	0	9	2	8	60	54	49	55	9	6	10	14	5	n/a	n/a
44	Yes	1	1	n/a	1	98	96	84	3	47	64	9	33	63	n/a	n/a
45	Yes	0	38	23	6	44	36	n/a	53	8	13	9	46	26	n/a	n/a

[a]IF : induction failure; [b]n/a : not available.