Detection of Minimal Residual Disease in Pediatric B Cell Acute Lymphoblastic Leukemia by Flow Cytometry

Pediatrik B Hücreli Akut Lenfoblastik Lösemide Minimal Kalıt Hastalığının Akan Hücre Ölçer ile Saptanması

Suzan ÇINAR,[a]
Abdullah YILMAZ,[a]
Umut Can KÜÇÜKSEZER,[a]
Günnur DENİZ[a]

[a]Department of Immunology,
İstanbul University,
Institute of Experimental Medicine,
İstanbul

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Yazışma Adresi/*Correspondence:*
Suzan ÇINAR
İstanbul University,
Institute of Experimental Medicine,
Department of Immunology,
İstanbul,
TÜRKİYE/TURKEY
scinar@istanbul.edu.tr

ABSTRACT Objective: Improvements in the treatment of childhood acute lymphoblastic leukemia (ALL) provide a complete remission in many patients. Several study groups indicated that determining submicroscopic levels of leukemia cells in the bone marrow (minimal residual disease-MRD) on day 15 is affects the prognosis. Flow cytometry (FCM) is a fast and cheap approach when compared to other molecular methods. In this study, day 15 bone marrow MRD levels and demographic profile in B-ALL patients assessed in our laboratory between December 2010 and August 2011 were discussed. **Material and Methods:** Bone marrow samples obtained on treatment day 0 and 15 from patients diagnosed with ALL (n=45) and the expressions of CD10, CD11a, CD19, CD20, CD34, CD38, CD45 and CD58 have been determined using FACSCalibur according to the Associazione Italiana Ematologia Oncologia Pediatrica- Berlin Frankfurt Münster (AIEOP-BFM) protocol and evaluated by CELLQuest-Pro software. After determination of the number of nucleated cells with Syto16, the percentages of leukemic cells (blast-MRD percentage) detected in the nucleated CD19+ B cell population were classified as flow low (FLR), flow medium (FMR) and flow high (FHR) risk. **Results:** Six of the 45 cases (10 females, 35 males, 6.17±3.90 years) in our survey were MRD negative and 39 were positive. Eleven cases were determined as FLR (24.4%), 26 as FMR (57.8%) and 8 as FHR (17.8%) according to MRD risk. **Conclusion:** All of the evaluations were approved by an AIEOP-BFM partner (100%). Since 30 August 2011, our institute has become the first center in Turkey to evaluate its own cases with the qualifications of our AIEOP-BFM partner (Vienna). In the future, we plan to investigate correlation of MRD-FCM results with prognosis and relapse, and to compare these findings with the polymerase chain reaction results.

Key Words: AIEOP protocol 8202; flow cytometry; leukemia, B-cell

ÖZET Amaç: Çocukluk çağı akut lenfoblastik lösemi (ALL) tedavisindeki ilerlemeler hastaların çoğunda tam remisyon sağlamaktadır. Farklı çalışma grupları tedavinin 15. gününde kemik iliğinde lösemi hücrelerinin submikroskobik düzeylerinin saptanmasının (minimal kalıt hastalık, MRD) prognoz ile ilişkili olduğunu göstermiştir. Flow sitometri (FCM), moleküler yöntemlerle karşılaştırıldığında daha hızlı ve ucuzdur. Günümüzde 100'den fazla merkezde uygulanmakta olan MRD-FCM yöntemi, lösemi tedavi eden farklı merkezlerden gönderilen örneklerde çalışmak üzere Türkiye'de ilk kez enstitümüzde başlatılmıştır. Bu makalede Aralık 2010-Ağutos 2011 tarihleri arasında laboratuarımızda değerlendirilen B-ALL olgularının 15. gün kemik iliği örneklerinde MRD düzeyleri ve demografik özellikleri tartışılmıştır. **Gereç ve Yöntemler:** AALL tanısı almış hastalardan (n=45) tedavinin 0. ve 15. gününde alınan kemik iliği örneklerinde CD10, CD11a, CD19, CD20, CD34, CD38, CD45 ve CD58 ekspresyonu FACSCalibur cihazı ile Associazione Italiana Ematologia Oncologia Pediatrica- Berlin Frankfurt Münster (AIEOP-BFM) protokolüne göre saptanmış, elde edilen veriler CELLQuest-Pro yazılımı ile değerlendirilmiştir. Çekirdekli hücre sayısı Syto16 ile belirlendikten sonra, çekirdekli CD19+ B hücre popülasyonu içinde saptanan lösemik hücrelerin tüm çekirdekli hücrelere oranı (% blast-MRD) hesaplanmış, flow düşük (FLR), orta (FMR) ve yüksek (FHR) risk olarak sınıflandırılmıştır. **Bulgular:** Çalışmamızdaki 45 olgudan (10 kız, 35 erkek, 6,17±3,90 yıl) altısı MRD negatif, 39 olgu ise pozitiftir. MRD risk skorlamasına göre 11 olgu FLR (%24,4), 26 olgu FMR (%57,8) ve 8 olgu FHR (%17,8) olarak saptanmıştır. **Sonuç:** Değerlendirmelerin hepsi AIEOP-BFM partner (Viyana) tarafından onaylanmış (%100) ve merkezimiz AIEOP-BFM partnerin denetiminde 30 Ağustos 2011 tarihinde yeterlilik kazanarak kendi olgularını değerlendirebilen Türkiye'deki ilk merkez olmuştur. Yeni çalışmalardan elde edilecek veriler doğrultusunda MRD-FCM sonuçlarının prognoz ve relaps ile ilişkisinin, polimeraz zincir reaksiyonu sonuçları ile uyumunun araştırılması hedeflenmektedir.

Anahtar Kelimeler: AIEOP protokolü 8202; akım sitometri; lösemi, B-hücre

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The progress in the therapy of childhood acute lymphoblastic leukemia (ALL) provides full remission in the majority of patients, and early response to the therapy is a prognostic factor.[1,2] Blast counts of peripheral blood on day 8 or bone marrow on day 15 are widely used for determination of risk-intended therapy.[3-5] Recent studies pointed out that determination of submicroscopic levels of leukemia cells (minimal residual disease, MRD) is feasible.[6] As revealed in the past 10 years, MRD levels forecast the prognosis of the patient when compared to traditional features, such as age and leukocyte count, and is nowadays frequently used for the estimation of different risk groups of patients at the first therapy stage.[7-11] Patients were recently divided into 3 risk groups according to the results of receptor gene rearrangements detected by real-time polymerase chain reactions (RT-PCR) within bone marrow samples, which were aspirated at the end of induction day 33 and day 78 phases.[12] Even though molecular determination of MRD is well-standardized, less-standardized flow cytometry (FCM) is faster, generally cheaper and provides data in a higher percentage of patients when compared with molecular techniques.[13-15] Single MRD-FCM detection of bone marrow samples on day 15 of the therapy can be converted and has a powerful prognostic capacity.

This detection is an early precursor of relapse and can be applied to almost all patients. PCR-MRD detection converted in later time phases can be helpful in the determination of supplemental therapies to suit each individual.[16] Assessment of MRD by FCM is based on repeatability of leukemic immunophenotyping defined in the diagnosis.[17] For the management of MRD, expression levels of 7 important antigens are investigated in the diagnosis of B-cell precursor ALL (BCP-ALL) and compared at different points of the therapy aimed for the induction of remission. The results show decreased CD10 and CD34 expressions, increased CD19, CD20, CD45RA and CD11a expressions, and non-changed CD58 expression levels. According to these findings, leukemic and normal residual B cells are precisely discriminated.[18]

Since initiation by a single institution, MRD-FCM is now carried out in more than 100 centers and applied to more than 2,000 patients.[19-23] For the first time, MRD-FCM determination is initiated in Institute of Experimental Medicine (DETAE) in Turkey with the aim of detecting MRD levels by FCM and supporting B-ALL care centers for patient follow-up, according to the protocol of "Associazione Italiana Ematologia Oncologia Pediatrica-Berlin Frankfurt Münster (AIEOP-BFM)".

MATERIAL AND METHODS

THE STUDY POPULATION

Forty-five patients (10 females and 35 males with a mean age of 6.17±3.90 years) diagnosed with ALL between 10 December 2010 and 22 August 2011 were enrolled in the study (Table 1).

The distribution of patients was as follows: 22 from Bakirkoy Education and Research Hospital (ERH; subsequently renamed Kanuni Sultan Suleyman ERH in June 2011), 11 from Goztepe ERH, 3 from Okmeydani ERH, 5 from Kocaeli University, Faculty of Medicine, 2 from Sisli Etfal ERH, 1 from İstanbul University Cerrahpasa Faculty of Medicine, 1 from Behcet Uz ERH. This study was done after obtaining the informed consent of the patients' parents or guardians.

Heparinized bone marrow samples were aspirated on day 0 of remission therapy and phenotype diagnosis (B-ALL) and the estimation of blast cells was done using FCM. On day 15, blast cells (MRD) were re-investigated.

FLOW CYTOMETRY

The Preparation and Labeling of Samples

Heparinized bone marrow samples were transferred to the laboratory within 4 hours of aspiration and immediately prepared for analysis. The nucleated cell count was determined using a RT-7600 hematology auto-analyzer (Rayto Life and Analytical Science Co. Ltd., Shenzhen, China). Blasts were labeled by anti-CD7, CD10, CD19, CD33, CD34, CD45 (all from BD Biosciences) monoclonal antibodies, using the "stain, lyse and then wash" approach. T and B-ALL panels were selected

TABLE 1: The age and gender profiles of subjects and minimal residual disease-flow cytometry findings.

Patient No	Gender	Age (Years)	Sample Quality	Normoblast %	Referring Hospital	MRD	MRD %	Risk	Blast /ml	Lymphocyte 10^3/ml	WBC 10^3/ml
1	M	6.41	D	0.18	Goztepe ERH	P	1.809	FMR	47.93	0.7	2.65
2	M	6.12	D	1.23	Okmeydani ERH	P	0.582	FMR	48.30	7.69	8.3
3	M	1.38	A	5.38	Goztepe ERH	P	0.006	FLR	0.19	2.39	3.17
4	M	3.84	A	2.82	Bakirkoy ERH	P	0.457	FMR	20.38	3.76	4.46
5	M	6.93	A	8.72	Bakirkoy ERH	**N**	0.018	FLR	0.81	3.86	4.51
6	F	0.67	A	2.00	Bakirkoy ERH	**N**	0	FLR	0	5.36	10.28
7	M	9.23	A	10.94	Bakirkoy ERH	P	32.312	FHR	2921.00	6.18	9.04
8	M	9.32	A	9.05	Kocaeli University MF	P	0.307	FMR	7.83	1.81	2.55
9	M	14.40	A	42.32	Okmeydanı ERH	P	1.569	FMR	143.56	5.76	9.15
10	M	7.09	A	3.06	Bakirkoy ERH	P	0.297	FMR	9.86	2.03	3.32
11	M	5.42	A	12.78	Kocaeli University MF	P	12.556	FHR	899.01	5.56	7.16
12	M	5.00	A	8.53	Kocaeli University MF	P	0.006	FLR	0.16	2.33	2.71
13	F	2.46	A	7.17	Bakirkoy ERH	**N**	0.002	FLR	0.12	2.56	5.97
14	M	3.49	A	2.35	Bakirkoy ERH	P	0.615	FMR	11.99	1.8	1.95
15	M	3.99	A	2.46	Bakirkoy ERH	P	13.772	FHR	296.10	1.93	2.15
16	M	14.34	A	23.24	Bakirkoy ERH	P	0.04	FLR	1.55	2.42	3.87
17	M	8.47	A	18.81	Bakirkoy ERH	**N**	0	FLR	0	2.08	4.34
18	M	2.50	A	2.73	Bakirkoy ERH	P	0.086	FLR	1.50	1.39	1.74
19	M	5.39	A	16.18	Bakirkoy ERH	P	1.804	FMR	85.87	4.06	4.76
20	M	6.96	A	3.00	Bakirkoy ERH	P	39.978	FHR	1815.00	3.71	4.54
21	F	1.63	D	0.72	Kocaeli University MF	P	1.46	FMR	58.84	2.99	4.03
22	M	3.89	A	27.94	Okmeydani ERH	P	27.373	FHR	3851.38	11.68	14.07
23	M	5.90	A	44.99	Cerrahpasa MF	P	0.013	FLR	23.77	104.98	182.85
24	M	5.22	A	17.51	Kanuni Sultan Suleyman ERH	P	3.087	FMR	45.38	1.17	1.47
25	M	16.47	A	28.92	Kanuni Sultan Suleyman ERH	**N**	0.015	FLR	0.53	2.35	3.51
26	M	7.25	A	18.11	Kanuni Sultan Suleyman ERH	P	1.038	FMR	94.04	6.51	9.06
27	M	5.47	A	34.36	Kocaeli University MF	P	8.750	FMR	819.88	5.99	9.37
28	M	13.89	A	5.51	Goztepe ERH	**N**	0.021	FLR	0.61	2.00	2.89
29	M	2.28	A	15.90	Goztepe ERH	P	0.430	FMR	-	-	-
30	M	10.85	A	8.39	Kanuni Sultan Süleyman ERH	P	0.667	FMR	61.50	3.61	9.22
31	F	12.20	A	5.89	Goztepe ERH	P	5.240	FMR	63.40	1.07	1.21
32	F	3.33	A	44.99	Goztepe ERH	P	0.080	FLR	3.34	2.6	4.18
33	M	4.67	A	35.31	Kanuni Sultan Süleyman ERH	P	0.910	FMR	104.10	6.74	11.44
34	M	5.28	A	18.17	Kanuni Sultan Süleyman ERH	P	0.149	FMR	9.48	5.14	6.36
35	F	-	A	46.29	Behcet Uz ERH	P	0.124	FMR	27.37	12.56	22.07
36	M	9.90	A	10.75	Sisli Etfal ERH	P	72.300	FHR	25876.17	29.65	35.79
37	M	4.00	A	31.50	Goztepe ERH	P	1.107	FMR	125.42	9.16	11.33
38	M	2.83	A	21.89	Kanuni Sultan Süleyman ERH	P	0.745	FMR	76.51	8.75	10.27
39	F	4.26	A	2.00	Kanuni Sultan Süleyman ERH	P	0.193	FMR	9.17	4.49	4.75
40	M	11.32	A	39.24	Sisli Etfal ERH	P	32.904	FHR	4659.21	8.88	14.16
41	F	3.26	A	25.93	Kanuni Sultan Suleyman ERH	P	3.409	FMR	187.50	3.79	5.5
42	M	3.09	A	10.70	Kanuni Sultan Suleyman ERH	P	0.876	FMR	26.28	2.25	3
43	F	0.31	A	6.55	Goztepe ERH	P	10.164	FHR	421.81	2.6	4.15
44	F	7.12	A	23.48	Goztepe ERH	P	1.675	FMR	208.87	7.77	12.47
45	M	5.68	A	33.08	Goztepe ERH	P	0.951	FMR	183.73	11.21	19.32

M: Male: F: Female; A: Adequate; D: Dilution; ERH: Education and research hospital; MF: Medical faculty; P: Positive; N: Negative; FLR: Flow low risk; FMR: Flow medium risk; FHR: Flow high risk.

in relation to over-expressed markers and for the B-ALL phenotype, CD10-fluorescein isothiocyanate (FITC), phycoeritrin (PE), PE-cyanin 7 (PEcy7), CD11a-PE, CD19-allophycocyanin (APC), CD20-FITC, CD34-PE, CD45-peridinin chlorophyll protein complex (PerCP) and CD58-FITC monoclonal antibodies were used (BD Biosciences, USA). Bone marrow samples were directly stained with four-color monoclonal antibody cocktails for 15 minutes at room temperature and in the dark. Following incubation, 2 milliliters of FACS lysing solution (BD Biosciences, USA, diluted at a ratio of 1/10 with distilled H_2O) was added and incubated for 10 minutes at room temperature and in the dark for the lysis of erythrocytes. Following the lysis step, cells were washed with 2 milliliters of PBS, re-suspended in 500 milliliters of PBS and subsequently analyzed using FCM.[21-24]

DATA ACQUISITION AND ANALYSIS

Data acquisition was converted with a dual-laser FACSCalibur Flow Cytometer running Cell Quest Pro software (BD Biosciences, USA). Light scatter properties, auto-fluorescent levels and compensations were adjusted with normal peripheral blood lymphocytes and accepted as reference.[24] For immunophenotyping, minimum 100,000 and for assessing MRD minimum 300,000 syto-positive events (cells) of 700,000 labeled cells were acquired. Cell-transitive, alive-cell nucleic acid fluorochrome Syto 16 (emission 518 nm, Molecular Probes-Invitrogen, Oregon,USA) was combined with CD19/CD45, the remaining non-nucleated erythroid cells, thrombocytes and debris (Syto 16^-) were excluded and nucleated cell counts (Syto 16^+) and the real MRD percentage were determined accordingly.[21]

CALCULATION

Data analysis was based on the determination of leukemic cells in a CD19 positive cell gate (Figure 1). Diagnosis was made with the determination of a minimum of 10 cell clusters showing an MRD leukemia-related immunophenotypic character (MRD positive). The blast ratio was determined. MRD risk below 0.1% was scored as flow low (**FLR**), risk between 0.1% and 10% as flow medium (**FMR**, Figure 1) and risk above 10% as flow high risk (**FHR**). The results were sent to partner AIEOP-BFM (Vienna) for approval.

SAMPLE QUALITY

The quality of bone marrow samples that arrived at the laboratory was defined by $CD45^-CD19^-$ cell (normoblast) ratios. Normoblast ratios above 2% were accepted as eligible whereas ratios below 2% were accepted as diluted because of peripheral blood contamination.

STATISTICAL INTERPRETATION

Statistical analysis of the experimental data was performed using a Mann Whitney-U test, Kruskall-

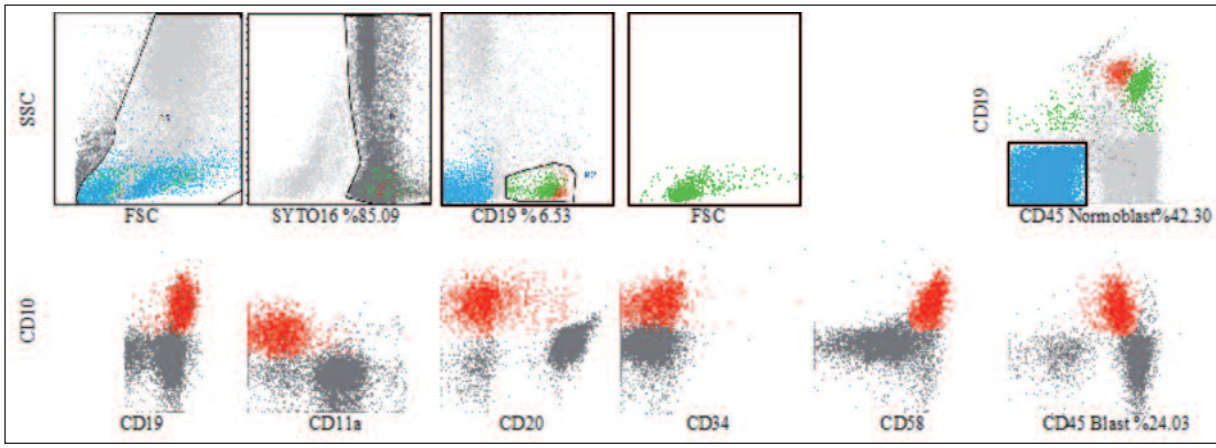

FIGURE 1: Representative flow cytometric dot plots from minimal residual disease- positive flow cytometry case (1.569%).
(See color figure at http://tipbilimleri.turkiyeklinikleri.com/)

Wallis test, Chi square test/Fisher's exact test and Freeman-Halton extension of Fisher's exact test with SPSS11.5 software. A value of $p<0.05$ was considered statistically significant.

RESULTS

There was a significant difference between the ages: mean ages were 6.72±3.82 (± standard deviation) years (5.68, 1.38-16.47) (median, min-max) in males and 4.03±3.60 (3.33, 0.67-12.20) years in females (p=0.027). When the sample quality was analyzed using percentage of normoblasts 10.75% (0.18-46.29), 93.3% of the samples (42/45) were found to be adequate 11.86% (2.00-46.29), predicted >2%, only 3 samples 0.72% (0.18-1.23), predicted >2% were suspected to be contaminated with peripheral blood.

Of the 45 patients (Table 1), 6 (13.3%) were found as MRD negative on the course of therapy (day 15) according to the calculated MRD risk, while the remaining patients were distributed among different levels of positivity: 12 (26.7%) had 0.01% to <0.1%, 25 (55.6%) had 0.1% to <10.0%, and 8 (17.8%) had ≥ 10.0%. According to different MRD levels, these patients were divided into three groups: Flow MRD low risk (FLR; MRD <0.1%), Flow MRD medium risk (FMR; 0.1% ≤MRD <10.0%) and Flow MRD high risk (FHR; MRD ≥10.0%, Table 2). All assessments were approved by AIEOP-BFM partner (Vienna). No significant difference was observed between the MRD risk groups regarding gender. The entire study group's bone marrow white blood cell (WBC) rate count was 4.75 (1.21-182.85) x 10^3/ml, lymphocyte count was 3.77 (0.70-104.98) x 10^3/ml, and blast count was 47.94 (0-25876.17) /ml. According to the FCM MRD risk score, blast counts were calculated as 0.57 (0-23.77)/ml for FLR subjects, as 58.84 (0-819.88)/ml for FMR subjects and finally as 2368.00 (296.10-25876.17) /ml for FHR subjects.

The difference of therapy responses between genders was statistically evaluated. No significant difference was found between MRD risk (FLR, FMR and FHR) and MRD percentages of the two gender groups (Table 2). All subjects were divided into 3 groups according to their ages (0-4.99, 5-9.99 and >10 years) and no significant differences were observed among the groups for therapy response (MRD%), 0.54% (0.00-27.37), 1.04% (0.00-72.30) and 0.67% (0.01-32.90), respectively (Krukall-Wallis p=0.608). According to the classical risk features such as age and diagnosis WBC, cases were divided into two groups: standard (1-9 years; <50000 WBC/ml) and high risk (<1 or >10 years or >50000 WBC/ml). The distribution of day 15 MRD-FCM risk regarding standard and high risk groups (Freeman-Halton extention of Fisher's exact test p=0.058) was summarized in Table 3.

TABLE 2: The minimal residual disease- flow cytometry findings of subjects in relevance with genders.

	Gender			
	F	M	Total	p
Subjects (n)	10	35	45	
Age (Year)	3.33 (0.67-12.20)	5.68 (1.38-16.47)	5.39 (0.67-16.47)	*0.027
MRD Positive n (%)	8 (80%)	31 (88.6%)	39 (87.7%)	**0.601
MRD Negative n (%)	2 (20%)	4 (11.4%)	6 (13.3%)	
FLR n (%)	3 (30.00%)	9 (25.67%)	12 (26.67%)	***0.798
FMR n (%)	6 (60.00%)	19 (54.33%)	25 (55.56%)	
FHR n (%)	1 (10.00%)	7 (20.00%)	8 (17.78%)	
MRD (%)	0.83 (0-10.16)	0.67 (0-72.30)	0.67 (0-72.30)	*0.677
Normoblast (%)	6.22 (0.72-46.29)	18.17 (0.18-44.99)	10.75 (0.18-46.29)	*0.171
Blast /µl	43.10 (0-421.81)	11.99 (0-25876.17)	47.94 (0-25876.17)	*0.657
Lymphocyte (10^3/µl)	3.39 (1.07-12.56)	2.35 (0.70-104.98)	3.77 (0.70-104.98)	*0.967

median, (minimum-maximum) or %; M: Male; F: Female; P: Positive; N: Negative; FLR: Flow low risk; FMR: Flow medium risk; FHR: Flow high risk;
*Mann Whitney-U test; **Fisher's exact test; *** Freeman-Halton extention of Fisher's exact test.

TABLE 3: Distribution of day 15 minimal residual disease-flow cytometry risk according to age and diagnostic white blood cell count (WBC) of the patients; standart risk (1-9 years; <50000 WBC/μl) and high risk (<1 or >10 years or >50000 WBC/μl).

	FLR	FMR	FHR	Total
Standart risk	4	16	2	22
	18.20%	72.20%	9.10%	
High risk	7	5	3	15
	33.30%	46.70%	20.00%	
Total	11	21	5	37
	29.73%	56.76%	13.50%	

FLR: Flow low risk; FMR: Flow medium risk; FHR: Flow high risk.

DISCUSSION

Bone marrows analyzed in our laboratory had a mean normoblast ratio greater than 2% (15.51±13.43%), indicating low peripheral blood contamination of the samples. Three out of 45 samples (6.67%) were contaminated with the peripheral blood, and all of them showed FMR risk. Contamination with peripheral blood complicates the scoring in FMR cases at the border of FLR-FMR, and the evaluation for the presence of blasts in FLR cases. Because of peripheral blood contamination, blast cell numbers in these kind of samples can be under-calculated.

Prucker et al. conducted a retrospective study in 896 childhood ALL cases who received treatment according to ALL-BFM protocol. They evaluated mortality and stated that infant age and female gender were independent from an increased death rate.[25] A study evaluating 163 patients with ALL-IC-BFM protocol states that the negativity of MRD at induction therapy day 33 has a link with an age of 1-5 years and WBC count lower than 20000/μl, non-T immunophenotype, good prednisone response and the absence of M3 morphology on day 15, but no links were shown between gender and hyperdiploidy or BCP-ALL and TEL/AML1 fusion.[13] Similarly in current study, no links were shown between MRD risk groups and response related to gender.

Two principal methods for MRD detection in childhood ALL are the molecular analysis of B-cell receptor gene rearrangements and the flow cytometric analysis of aberrant immunophenotypes, both methods being predictive of outcome.[26,27] Both methods enabled the detection of one leukemic cell among at least 10^4 normal cells.[21] It can be sensitive more than 100 times compared to morphologic examination.[28] Published studies indicate that the analysis of insistent MRD indicators by flow cytometry can be a powerful prognostic factor. This approach can be applicable for many patients, but interaction with regenerating normal but immature lymphocytes should be evaluated with great care.[21,29] Hence, detection of MRD may have a profound impact on future clinical management strategies. The Berlin-Frankfurt-Münster (BFM) international cooperative study group has considered this development by basing the general stratification procedure in their recently issued treatment protocol (ALL-AIEOP/BFM 2000) on results of molecular genetic MRD assessment.[16, 26] In a report from the UK Flow MRD group, a network of 6 UK laboratories, which have validated a standardized protocol for B-lineage ALL, showed that this protocol had high sensitivity and technical applicability, good concordance with the gold standard molecular-based analysis and importantly, and was highly reproducible between laboratories across different instrument platforms.[27] In a Swedish multicenter study of childhood ALL, the MRD levels were analyzed in 726 follow-up samples of 228 children using real-time quantitative polymerase chain reaction (RQ-PCR) and FCM between 2002 and 2006, and concluded that the concordance between RQ-PCR and FCM was high and hence, both methods are valuable clinical tools for identifying childhood ALL cases with increased risk of relapse.[30]

After Ozbek et a. investigated TEL and AML1 translocation using the reverse transcription-polymerase chain reaction in patients with ALL as a prognostic indicator, MRD with FCM was started with cooperation of AIEOP-BFM partner laboratory in Vienna (St Anna Children's Cancer Research Institute) at September 2009.[31] After training, all results were sent by e-mail and then controlled and approved by AIEOP-BFM partner. Our center received a certificate of proficiency for

B-ALL on August 30, 2011. The DETAE Department of Immunology was the first center in Turkey that received proficiency and was able to evaluate the cases by itself.

In this report, according to the AIEOP-BFM protocol, only a small series, 45 cases, were analyzed. Our institute has become the first center in Turkey that evaluates its own cases with the qualifications of our AIEOP-BFM partner. The next aim of our center is to clarify the relationship between MRD-FCM results and prognosis-relapse, and also the concordance with PCR results.

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