Minimal residual disease (MRD) detection with translocations and T-cell receptor and immunoglobulin gene rearrangements in adult acute lymphoblastic leukemia patients: a pilot study

Yetişkin akut lenfoblastik lösemili hastalarda translokasyonlar ile T-hücre reseptörleri ve immunglobulin yeniden yapılanmalarını kullanarak minimal rezidüel hastalık tespiti

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Abstract

Objective: Monitoring minimal residual disease has become increasingly important in clinical practice of ALL management. Break-point fusion regions of leukaemia related chromosomal aberrations and rearranged immunoglobulin (Ig) and T cell-receptor (TCR) genes are used as leukaemia specific markers in genetic studies of MRD.

Material and Methods: A total of 31 consecutive patients with newly diagnosed ALL were screened for eligibility criteria. Of those 26 were included in the study. One patient with partial response following induction therapy and four patients who were lost to follow-up after induction were excluded from the study; thus 21 patients were evaluated for MRD by using polymerase chain reaction (PCR), heteroduplex analysis, sequencing and quantitative real time PCR techniques.

Results: Chromosomal aberrations were detected in 5 (24%) of the patients and were used for MRD monitoring. Three patients had t(9;22) translocation, the other 2 had t(4;11) and t(1;19). MRD-based risk stratification of the16 patients analysed for Ig/TCR rearrangements revealed 3 low-risk, 11 intermediate-risk and 2 high-risk patients.

Conclusion: MRD monitoring is progressively getting to be a more important predictive factor in adult ALL patients. As reported by others confirmed by our limited data there is a good correlation between MRD status and clinical outcome in patients receiving chemotherapy. The pilot-study presented here is the first that systematically and consecutively performs a molecular MRD monitoring of ALL patients in Turkey. *(Turk J Hematol 2008; 25: 124-32)*

Key words: MRD monitoring, Ig/TCR gene rearrangement, ALL.

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Özet

Amaç: Minimal rezidüel hastalığın (MRH) takibi, ALL tedavisinin klinik uygulamalarında giderek önem kazanmaktadır. Lösemide sıklıkla görülen kromozomal kırık noktaları ve immunglobulin (Ig) ve T-hücre reseptörleri (THR), MRH takibinde lösemiye özgü marker olarak kullanılmaktadır.

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Gereç ve Yöntemler: Yeni ALL tanısı almış toplam 31 hasta çalışmaya alındı, kriterlere uygun 26 hasta ile çalışma gerçekleştirildi. 5 hasta çalışma sürecinde takip dışı kaldı ve 21 hasta polimeraz zincir reaksiyonu (PCR), heterodupleks analizi, dizileme ve kantitatif "real time" PCR yöntemleri kullanılarak MRH açısından değerlendirilmeye alındı.
Bulgular: Bu hastalardan 5 (%24) tanesinde kromozomal translokasyonlar tespit edildi. 3 hastada t(9;22), 1 hastada t(4;11) ve 1 hastada da t(1;19) translokasyonları belirlendi. MRH'a dayalı risk tespitinde 16 hasta Ig/THR yeniden yapılanmaları için analiz edildi ve 3 tanesi düşük risk gurubu, 11 tanesi orta risk gurubu ve 2 tanesi de yüksek risk gurubuna dahil edildi.
Sonuç: Elde ettiğimiz sonuçlar, kemoterapi alan hastalarda klinik sonuçların, MRH durumları ile iyi korelasyon gösterdiğini desteklemektedir. Bu pilot çalışma ile ülkemizde ilk defa sistematik olarak tanı ve takip örneklerinde MRH takibi gerçekleştirilmiştir. *(Turk J Hematol 2008; 25: 124-32*)
Anahtar kelimeler: MRH takibi, Ig/THR gen yeniden düzenlenmesi, ALL.

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Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous malignant disease of lymphoid hematopoietic precursor cells. Complete remission (CR) rates of up to 95% can be achieved with current treatment strategies in ALL. However, responses are not durable and relapse is inevitable for most ALL patients, resulting in long-term survival rates of only 30-35% [1-4]. Residual malignant cells that remain beyond the detection level of standard cytomorphological diagnostic methods are held responsible for the ultimately ensuing relapse. These cells represent the minimal residual disease (MRD). Thus, monitoring MRD has become increasingly important in clinical practice of ALL management. A large number of studies have shown that MRD status in hematopoietic malignancies is a useful tool for predicting clinical outcome [4]. Techniques for studying MRD rely on the detection of a leukemia cell-specific marker that enables distinguishing blasts from normal cells. Currently, two methods are available: [1] genetic (molecular) studies and [2] immunophenotyping.

Break-point fusion regions of leukemia-related chromosomal aberrations and rearranged immunoglobulin (Ig) and T–cell receptor (TCR) genes, which can be detected by polymerase chain reaction (PCR), are used as leukemia-specific markers in genetic studies of MRD. Recurrent chromosomal aberrations are detected in 30-40% of ALL cases [5]. The four most commonly encountered chromosomal translocations are t(9;22), t(12;21), t(4;11) and t(1;19). Newly developed real-time quantitative PCR techniques are sensitive to detect one leukemic cell in10^4-10^6 normal cells [5].

The immune system encounters millions of different antigens and antigenic epitopes. A limited number of gene segments are able to code for the receptor diversity by the combinations of gene segments, which are different in each lymphocyte or lymphocyte clone. During early differentiation of B- and T-cells, the germ-line segments of variable (V), diversity (D) and joining (J) genes in Ig and TCR gene complexes rearrange. Each lymphocyte gets a specific combination of V-(D)-J segments that codes the variable domains of Ig or TCR molecules [6]. The random insertion and deletion of nucleotides at the junction sites of V, (D), and J gene segments turn the junctional regions of Ig and TCR genes into fi gerprint-like sequences, which probably differ in each lymphocyte and thus in each lymphoid malignancy. Therefore, junctional regions can be used as leukemia-specific markers in the analysis of MRD [7]. Such targets can be identified at initial diagnosis by various PCR primer sets in >95% of patients with lymphoid malignancies [6,8]. Immunological MRD tests include the flow-cytometric profiling of the leukemia-associated aberrant immunophenotype [9,10]. Results from MRD analysis based on flow-cytometric immunophenotyping were found similar to those of molecular studies [11].

Several studies based on Ig/TCR gene rearrangements for monitoring MRD have shown that the most important application of MRD monitoring in ALL is to assess initial responses to cytotoxic therapy [12]. After the induction therapy, low amounts or absence of MRD in bone marrow seem to predict outcome, and the risk of relapse is proportional to the level of MRD [13,14]. Multivariate analysis showed that the level of MRD after induction therapy is the most powerful prognostic factor, independent of other clinically relevant risk factors, such as age, blast count, immunophenotype, presence of chromosomal aberrations at diagnosis, and response to prednisone [15]. The results from the large prospective MRD study of the international BFM Study Group showed that the information on the kinetics of the decrease in tumor load after induction treatment and before consolidation treatment is more useful than analyzing MRD at one time point [13].

Accordingly, patients can be separated into three subgroups as: 1 Low-risk patients (no MRD at both time points, 5-year relapse rate of 2%); 2 high-risk patients (with intermediate 10^{-3} or high $\geq 10^{-2}$ MRD at both time points, 5-year relapse rate of 80%); and 3 intermediate-risk patients (5-year relapse rate of 22%).

Due to financial and technical constraints, molecular MRD monitoring of ALL has been a "difficult-to-perform" task in Turkey and is still not a routine laboratory procedure. The primary aim of this pilot study was to establish the molecular method of MRD monitoring in ALL and create the required technical background for a central MRD follow-up in Turkey. The secondary aim was to determine the impact of MRD on clinical outcome of ALL.

Material and Methods

Patients

All patients referred to the Hematology Outpatient Clinic of Cerrahpaşa Medical Faculty with the probable diagnosis of ALL between March 2002 and December 2004 were screened for

Table 1. Conventional risk stratification in adult ALL

	Good (Standard)	Adverse (High)
Age	≤ 50 years	> 50 years
WBC	≤ 30000/μl	> 30000/μl (in B-lineage)
	≤ 100000/μl	> 100000/μl (in T-lineage)
Immunophenotype	Thy ALL	Pro B [B-lineage, CD10 (-)]
	Early T (T-lin., CD1a -, sCD3-)	
	Mature T (T-lin., CD1a -, sCD3+)	
Cytogenetics	High hyperdiploidy	
Molecular genetics	Complex aberrations)	TEL-AML
		t(9;22)/BCR-ABL
		t(4;11)/ALL1-AF4
		t(1;19)/PBX-E2A

eligibility. Patients with newly diagnosed ALL of any type (except mature B-ALL –L3) who were 15 years of age or older and had adequate bone marrow samples for molecular MRD studying were included in the study. Morphological and immunophenotypical diagnoses of ALL were made according to French-American-British (FAB) [16] and the European Group for Immunological Classification of Acute Leukemia (EGIL) [17] criteria, respectively. Only the patients who achieved morphological complete remission (mCR) following induction therapy were evaluated for MRD monitoring.

All patients were treated with the GMALL 93/05 chemotherapy protocol [18]. Treatment consisted of an induction, reinduction, and consolidation therapy lasting one year based on different combinations of the drugs methylprednisolone, cyclophosphamide, vincristine, daunorubicin, methotrexate, asparaginase, cytosine arabinoside, 6-mercaptopurine (6-MP), and etoposide (VP16). The maintenance therapy consisted of intermediary application of 6-MP plus methotrexate for a total of two years. The combination, dosage and schedule of the chemotherapy differed for T-ALL, and standard- and high-risk B-ALL [19]. A search for HLA-matched donor was initiated at first clinical/morphological relapse. The study protocol allowed patients aged less than 46 and with a good performance status to proceed to allogeneic bone marrow transplantation if they had achieved a second mCR and had a fully-matched donor.

Bone marrow samples for morphological, immunophenotypical and molecular analyses were collected from all patients at initial diagnosis and then from those who achieved a post-induction CR at pre-defined time points (Days (D) 30, 90, 180, 270, 360) during the remainder of the therapy. Additional yearly MRD controls after D360 were done only for those patients who maintained mCR.

Table 2. MRD-based risk grouping in ALL

MRD risk group	After Induction	Post Consolidation
MRD low risk	$<10^{-4}$ and negative	always $< 10^{-4}$
MRD high risk	$> 10^{-4}$ or	2 times $> 10^{-4}$
MRD intermediate risk	MRD evaluation not possible	
	Technical prerequisites not fulfilled	
	Inconclusive course of MRD	

Note: Technical prerequisites are: at least two clone specific markers with minimum sensitivity of 10^{-4}; material from decisive timepoints available

The treatment, clinical management, follow-up and immunophenotyping of the patients as well as the morphological examination of bone marrow samples were done in the Hematology and Pathology Departments of Cerrahpaşa Medical Faculty, İstanbul University. Laboratory work of molecular MRD monitoring was performed in the Genetics Department of the Institute for Experimental Medicine and Research (DETAE), İstanbul University.

Patients were also classified according to conventional and MRD-based risk grouping systems (Tables 1, 2) [20].

The study was approved by the ethical committee of the Cerrahpaşa Faculty of Medicine, İstanbul University, and informed consents were obtained from all the participating patients.

Methods

The initial marrow samples were first analyzed for the presence of four unique chromosomal translocations, namely t(4;11), t(9;22)-p190, t(9;22)-p210, t(12;21) and t(1;19). For this, RNA was isolated by RNeasy isolation kit (Qiagen, GmbH Germany). 1μg of RNA, 1U of MMLV reverse transcriptase and random hexamer were used to synthesise cDNA. If the patient was found positive for one of the above-mentioned translocations in the initial (D0) sample, then the MRD monitoring was done using this marker thereafter.

If the patient was negative for all of the translocations, targeting PCRs were performed to find a patient-specific Ig/TCR rearrangement (Figure 1). In brief, genomic DNA was extracted from peripheral blood by using proteinase K/salting out method. For each 50μl PCR reaction, 60 ng DNA sample, 6.3 pmol of the primers, and 0.5 U Ampli Taq Gold polymerase (Applied Biosystems, Foster City, CA, USA) were used. The sequences of oligonucleotides used for amplification are given in Table 3. PCR conditions were 10 min at 94°C, followed by 35 cycles of 45 sec at 92°C, 90 sec at 60°C, and 2 min at 72°C, and last step of 10 min at 72°C (Applied Biosytems 9600). Positive and negative controls were included in all experiments.

PCR products had to be analyzed for their clonal origin to confirm that they were derived from the malignant cells and not from contaminating normal cells with similar Ig or TCR gene rearrangements. For this, precise nucleotide sequences of the junctional regions were determined. This sequence information allowed the design of junctional region-specific oligonucleotides. These oligonucleotides were then used to detect malignant cells among normal lymphoid cells during follow-up of patients in two different ways. First, oligonucleotides were utilized as patient-specific junctional region probes in hybridization experiments to detect PCR products derived from the malignant cells. Second, the junctional region-specific oligonucleotides were used as primers to specifically amplify the rearrangements of the malignant clone.

To determine the clonality, PCR products were analyzed by heteroduplex gel technique. The samples were denatured at 94°C for 5 min after the final cycle of amplification and subsequently cooled to 4°C for 60 min to induce duplex formation. Samples were then loaded immediately on 6% non-denaturing polyacrylamide gels in 0.5x tris-borate-EDTA (TBE), ran at room temperature, and visualized by ethidium

Table 3. Oligonucleotide sequences used for amplification

Primer code	Size of primer (bp)	Sequence (5'→3')
V_4-2T	18	tgggatgggcagagcaat
V_.7-2T	21	tgtgctagcagcttccctgag
V_3-6B	23	gggtctattactgccgaaggact
Intron-6B	16	gctttccgcccggagt
V_2-9B	17	gacggcccgtagggtgt
V_2-9B	18	gtgacacctcccggtgct
V_12.4-10T	22	tagcgccaggggtactactacg
VH4.4-11B	21	gcgagaggtggtgttcatgt
VH3.33-11B	17	gcggtggggggtattgt
J_30-11B	21	gttcccatatcccccagtacc
V_2-12B	20	cacctgggacccgaattatt
VH3.7-12B	24	gtgtattactgtgcgttcctggac
D_3-14B	18	cgtatcccccatcggaag
VH3.7-14B	24	gatacagctatgtagccccggtac
J_29-14B	21	caagaggtgtgtttcccctgg
V_1.17-15B	23	cataatagttaccctcggcccta
VH3.23-15B	22	ccggggactgactacgattct
V_2.3-15B	21	gcacacaagtcttgtcggagc
D_2-24B	21	taacattgtggggaccaccat

bromide staining. To confirm the clonality and to avoid the background positive control, a buffy coat, representing five healthy individuals, was used in each run. Monoclonal samples were used for the direct sequencing. The two bands of the biclonal samples were separated and removed from the polyacrylamide gel and the bands were sequenced separately.

Direct sequencing of rearrangements was performed with the specific primers using the dye-terminator cycle sequencing kit on an ABI377 sequencer as previously described [21]. Vd2-Dd3 sequences were evaluated for the presence of the common stems and segments, which were identified by comparison to germ-line sequences as previously described [22,23]. For alignments of Dd2 and Dd3 segments in Vd2-Ja junctional regions, at least five consecutive matching nucleotides were required. V_H, D_H and J_H segments were identified using DNAPLOT software (W. Müller H-H. Althaus, University of Cologne, Germany) by searching for homology with all known human germ-line V_H, D_H and J_H sequences obtained from the VBASE directory of human Ig genes. Vg and Jg gene segments were identified by comparison to germ-line gene TCRG sequences.

Patient-specific primers were positioned at the junctional regions. A standard annealing temperature of 60°C was used. To determine the efficacy of amplification and the sensitivity of PCR target, D0 sample was diluted in 10-fold steps into control steps from 10^{-1} down to 10^{-6}. Serial dilutions of diagnostic samples were analyzed in duplicate. For the correction of quantity and quality of DNA, albumin gene was used. Non-specific amplification was defined as any amplification observed in control DNA. The reproducible sensitivity of primers/probe combination was defined as the maximal 10-fold dilution step with a maximal difference in cycle threshold (C_T) value of 1.5 between the duplicate of involved dilution samples and with a maximal C_T value of 40 cycles. Furthermore, the C_T values of the reproducible sensitivity had to be at least three cycles lower than the C_T values of control DNA. If non-specific amplification was not observed, maximal sensitivity was defined as the maximal 10-fold dilution of the diagnostic sample giving specific but non-reproducible amplification.

MRD levels were stated as the proportion of leukemic cells to all nucleated cells in the sample. The aim was to obtain at least two molecular markers per patient with a sensitivity of min 10^{-4}. PCR results were interpreted according to the common criteria of the laboratories organized in the European Study Group on MRD-Detection in ALL (ESG MRD-ALL; coordinator: J. J .M. van Dongen, Rotterdam, The Netherlands).

Overall survival was estimated by using Kaplan-Meier analyses.

Results

A total of 31 consecutive patients with newly diagnosed ALL were screened for eligibility criteria. Of those, 26 were included in the study. One patient with partial response following induction therapy and four patients who were lost to follow-up after induction were excluded from the study; thus, 21 patients were evaluated for MRD (Table 4).

Mean age of the patients was 32 years (16-67). Of the 21 patients, 10 were female and 11 were male. Six patients (28%) had T-cell ALL and 15 patients (72%) were diagnosed as B-cell ALL. Mean leukocyte count and serum lactate dehydrogenase (LDH) levels at diagnosis were 54700/mm^3 (400-257000) and 1330 U/L (290-11754), respectively.

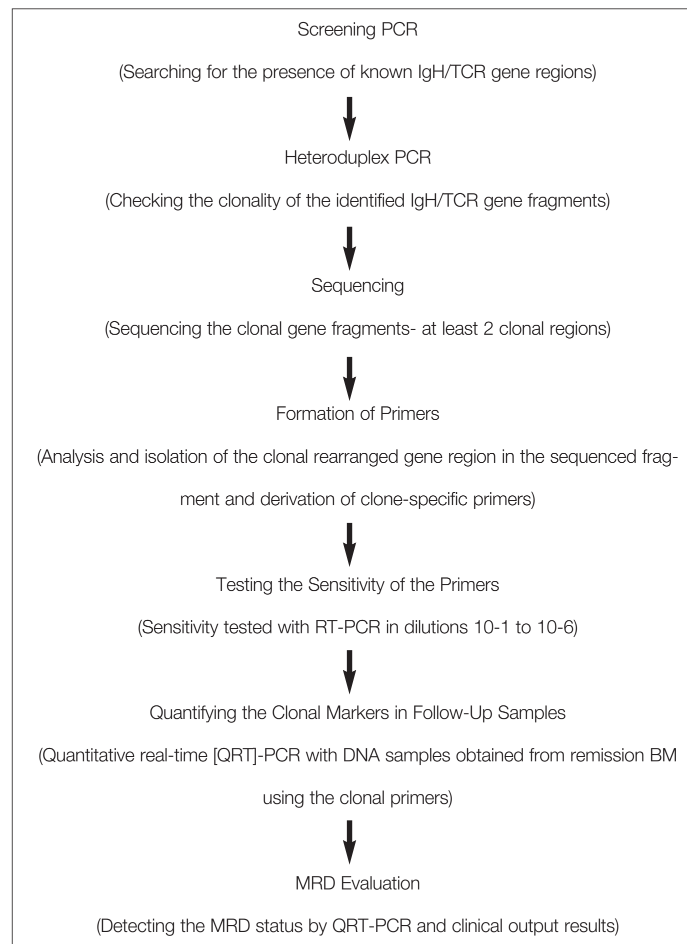

Figure 1. Flowchart of Ig/TCR rearrangement analysis

Table 4. Clinical and laboratory characteristics of the study patients

PI/Sex	Age	IP	WBC count (/µl)	LDH (U/L) (N<480)	Blast % (BM/P)	CG	EMD	mCR	CRG	Last Status
NY	31 F	T-ALL	4300	290	30 (BM)	(-)	(-)	(+)	S	Alive, in remission (63 months)
RA	19 M	T-ALL	51300	11754	99 (BM)	(-)	(-)	(+)	S	Alive, in remission (63 months)
SU	21 M	cB-ALL	600	342	98 (BM)	(-)	(-)	(+)	S	Died (14 months)
OE	28 M	B-ALL	100000	3713	90 (P)	t(1;19)	(-)	(+)	H	Died (3 months)
MT	32 M	T-ALL	58700	U	34 (P)	(-)	(-)	(-)	S	Unknown*
HK	56 F	cB-ALL	7400	1329	36 (BM)	(-)	(+)	(+)	S	Died (49 months)
ÖÖ	67 M	cB-ALL	70000	355	95 (BM)	t(9;22)	(-)	(+)	H	Died (14 months)
HA	32 F	T-ALL	1300	900	41 (BM)	(-)	(-)	(+)	S	Died (28 months)
GG	22 M	B-ALL	160000	2024	67 (BM)	(-)	(+)	(+)	H	Died (14 months)
ÇÖ	24 F	T-ALL	24000	U	84 (BM)	(-)	(-)	(+)	S	Died, in remission (5 months)
YD	16 M	B-ALL	400	261	58 (BM)	(-)	(+)	(+)	S	Died (33 months)
SA	43 F	pB-ALL	6400	2220	85 (BM)	(-)	(+)	(+)	H	Died (22 months)
NA	17 M	cB-ALL	6100	223	96 (BM)	(-)	(-)	(+)	S	Died (37 months)
BÇ	21 F	B-ALL	48000	738	80 (BM)	t(9;22)	(-)	(+)	H	Died (14 months)
SA	27 M	cB-ALL	3000	2969	90 (BM)	(-)	(-)	(+)	S	Alive, in remission (41 months)
AÇ	42 M	pB-ALL	30000	639	71 (BM)	(-)	(-)	(+)	H	Alive, in remission (41 months)
FÇ	45 M	pB-ALL	20400	U	U	(-)	U	U	H	Unknown*
AY	16 M	cB-ALL	U	U	U	(-)	(+)	(+)	U	Unknown*
MÇ	45 F	pB-ALL	257000	700	71 (BM)	t(4;11)	(-)	(+)	H	Died (16 months)
HA	37 F	ppB-ALL	4200	681	90 (BM)	(-)	(-)	(+)	H	Died (5 months)
ZC	74 F	cB-ALL	U	U	U	(-)	U	U	U	Unknown*
ST	22 M	T-ALL	81000	1141	62 (BM)	(-)	(+)	(+)	S	Alive, in remission (53 months)
ÖA	16 F	cB-ALL	2500	2489	70 (BM)	(-)	(-)	(+)	S	Alive, in active disease (35 months)
ŞS	46 F	pB-ALL	37000	U	U	(-)	(-)	U	H	Unknown*
TE	44 M	cB-ALL	46500	2450	96 (BM)	t(9;22)	(-)	(+)	H	Died (7 months)
AY	41 M	T-ALL	150000	2304	95 (BM)	(-)	(-)	(+)	H	Died (6 months)

PI: Patient initials, **IP:** Immunophenotype, **M:** Male, **F:** Female, **WBC:** White blood cell, **EMD:** Extramedullary disease, **CRG:** Conventional risk group, **H:** High risk, **S:** Standard risk, **U:** Unknown, **P:** Peripheral blood, **BM:** Bone marrow, **LDH:** Lactate dehydrogenase, **CG:** Cytogenetics, **mCR:** Presence of morphological complete remission following induction chemotherapy, **cB-ALL:** Common B-cell ALL, **pB-ALL:** ProB-cell ALL, **ppB-ALL:** Pre-proB-cell ALL, * Patients were not considered for MRD analysis due to lack of adequate clinical and laboratory data (see text for details)

Table 5. Characteristics of patients with chromosomal aberrations

Patient Initials	Age	Sex	Diagnosis	Translocation	D0	D30	D90	D180	D270	D360	Survival (months)
OE	28	M	B-ALL	t(1;19)	(+)	(+)	NA	-	-	-	3
ÖÖ	67	M	B-ALL	t(9;22)-p210	(+)	(-)	NA	NA	(+)	ND	14
BÇ	21	F	B-ALL	t(9;22)-p210	(+)	(+)	NA	(+)	ND	ND	14
MÇ	45	F	B-ALL	t(4;11)	(+)	(+)	(-)	(-)	(-)	(+)	16
TE	44	M	B-ALL	t(9;22)-p190	(+)	(-)	NA	(+)	-	-	7

NA: Not available (due to technical reasons). **ND:** Not done (the patient had morphologically active disease). **M:** Male. **F:** Female

The median follow-up was 22 months (3-63 months). Three-year survival rate was 38%. At the time of the last evaluation (July 2007 [36 months after the completion of last patient accrual]), six patients (29%) were alive. Five (3 T-ALL, 1 common-B-ALL and 1 precursor B-ALL) out of those six patients were still in CR with a median survival of 53 months and one was in late relapse. None of the long-term survivors exhibited bad prognostic chromosomal aberrations. About 25% of patients died within six months of diagnosis, following a post-induction CR, mainly due to infections and early relapse. Only one patient died in remission (5%) as a result of treatment-related genitourinary infection. According to the prognostic criteria of the German Acute Lymphoblastic Leukemia Group, 10 patients fulfilled the high-risk criteria, whereas the remaining 11 were in the standard-risk group (Table 4). Median survival in the standard-risk group was 37 months (5-63 months), with 7 of the 11 patients (63%) surviving more than three years. The median survival and the three-year survival rate in the high-risk group were significantly lower (14 months [3-41 months] and 10%, respectively).

Chromosomal aberrations were detected in 5 (24%) of the patients and were used for MRD monitoring. Three patients had t(9;22) translocation, and the other 2 had t(4;11) and t(1;19). By definition, all of these patients were at high-risk. Median duration of survival among these patients was 14 months (3-16 months) (Table 5).

Ig/TCR rearrangements were studied for the remaining 16 patients as MRD marker (Table 6). However, clonal Ig/TCR rearrangements could only be detected in seven of those patients. Since the number of patients was too low to do a statistical analysis, results were evaluated as case-based data:

-Patient RA had mCR and no detectable MRD at D30. His MRD continued to be negative at the end of the third year. No MRD was checked thereafter. The patient was still in mCR at the time of the last follow-up (63 months after diagnosis),

-Patient HK became MRD-negative by the end of the first year of her diagnosis while she was still receiving the pre-maintenance chemotherapy. She remained MRD-negative until the 21st month of treatment (oral maintenance phase) when an MRD of 6, E-05 was detected. Central nervous system (CNS) and systemic relapse soon followed. No donor search was initiated due to advanced age of the patient. She died of progressive disease 25 months after the relapse despite having had secondary chemotherapy regimens with considerable success.

-Patient GG, although having achieved a morphological remission after the induction chemotherapy, never became MRD-negative and showed a morphological relapse in the 6th month of therapy. He had no HLA-matched donor. He survived for another eight months on salvage chemotherapy regimens and died in sepsis 14 months after his diagnosis.

-Patient YD was a standard-risk patient who became MRD-negative just after the induction therapy and completed his chemotherapy schedule preserving his MRD negativity for about 20 months. He relapsed in the third year and died due to infection after a total survival of 33 months. No HLA-matched donor could be found.

-Patient SA never became MRD-negative but remained in clinical/morphological remission while receiving chemotherapy. Neither related nor unrelated HLA-matched donor was available. She relapsed in the period between the last two consolidation treatments. Thereafter, the patient was treated with a salvage regimen that resulted in CNS involvement. She then refused any kind of further chemotherapy and was followed with supportive treatment until she died of infection and bleeding 22 months after her diagnosis.

-Patient AC became MRD-negative within six months of diagnosis while still receiving the pre-maintenance phase of chemotherapy. No other MRD control could be done after the first year due to difficulties in follow-up. The patient was last seen in July 2007 (41 months after diagnosis), still in complete morphological and clinical remission.

-Patient OA, despite becoming MRD-negative at the D30 evaluation, became positive within six months of diagnosis. She remained in mCR while receiving consolidation chemotherapy. She ultimately relapsed after 18 months of treatment while on oral maintenance and survived on secondary chemotherapy regimens without achieving durable mCR. Thirty-five months after diagnosis she was still alive with active disease and receiving palliative treatment. No HLA-matched donor could be identified.

MRD-based risk stratification of the 16 patients analyzed for Ig/TCR rearrangements revealed 3 low-risk, 11 intermediate-risk and 2 high-risk patients. The overall survival rates were 35, 37, 18 for the low-, intermediate- and high-risk groups, respectively (Table 6).

GMALL 05/93 consists of a 52-week pre-maintenance phase including induction, reinduction and consolidation chemotherapies and an oral maintenance phase of two years. Nine of the 21 patients (43%) including the six long-term survivors were able to complete the pre-maintenance phase of the therapy. The median duration of treatment for this group was 100 weeks (almost twice as long as suggested in the protocol).

Discussion

Evidence-based data reported over the last years strongly indicate the importance of MRD monitoring in acute leukemias of adults and children. To date, there are two primary methods of MRD monitoring: immunophenotyping and molecular (genetic) studies. Both of these techniques have been studied extensively in adult ALL and were approved as valuable tools for relapse prediction. Since durable remission and long-term survival rates are less satisfying in adult ALL than in childhood ALL, MRD detection becomes much more important for the former. In Turkey, regular MRD monitoring for ALL is not a routine procedure except in some individual cases with known translocations.

Table 6. Characteristics of patients with Ig/TCR rearrangements

PI	Age	Sex	Dx	Ig/TCR Rearrangement	TS	D0	D30	D90	D180	D270	D360	MR	Survival (months)
NY	31	F	T-ALL	No clonal rearrangement could be detected								I	63*
RA	19	M	T-ALL	Vg4-Jg2.3 Vb7.7-Db1-Jb1.5	10^{-5} 10^{-5}	1,E+00	Neg.	Neg.	Neg.	Neg.	Neg.	L	63
SU	21	M	B-ALL	No clonal rearrangement could be detected								I	14*
HK	56	F	B-ALL	Vg3-Jg2.3 Intron-Kde	10^{-3}	1,E+00	NA	8,E-02	NA	Neg.	Neg.	I	49
HA	32	F	T-ALL	No clonal rearrangement could be detected								I	28*
GG	22	M	B-ALL	Vg2-Jg1.3 Vd2-Dd3	10^{-4} 10^{-4}	1,E+00 1,E+00	2,E-02 2,E-02	Neg. 2,E-04	1,E+02 1,E+02	NA	NA	H	14
ÇÖ	24	F	T-ALL	Vb12.4-Db1-Jb2.7		MRD monitoring could not be done due to inadequate follow-up material						I	5*
YD	16	M	B-ALL	Vd2-Dd3-Ja30 Vh3.33-Dh2.2-Jh6 Vh4.4-Dh2.21-Jh5b	10^{-4} 10^{-3}	1,E+00	Neg.	Neg.	Neg.	NA	Neg.	L	33
SA	43	F	B-ALL	Vg2-Jg2.3 Vh3.7-Dh2.2-Jh6	10^{-4} 10^{-4}	1,E+00	NA	NA	2,E-04	NA	2,E-02	H	22
NA	17	F	B-ALL	No clonal rearrangement could be detected								I	37*
SA	27	M	B-ALL	Vg9-Jg2.3 Vd2-Dd3 Vh3.7-Dh5.5-Jh4b Vd2-Dd3-Ja29 Vd2-Dd3-Ja58	 10^{-3} 10^{-4}	MRD monitoring could not be done due to inadequate follow-up material						I	41
AÇ	42	M	B-ALL	Vk1.17-Kde Vh3.23-Dh4.17-Jh4b Vk2.3-Kde	10^{-4}	1,E+00	6,E-02	4,E-02	Neg.	NA	Neg.	I	41*
HA	37	F	B-ALL	No clonal rearrangement could be detected								I	5*
ST	22	M	T-ALL	No clonal rearrangement could be detected								I	53*
ÖA	16	F	B-ALL	Db2-Jb2.7	10^{-4}	1,E+00	Neg.	NA	5,E-04	2,E-06	NA	L	35
AY	41	M	T-ALL	No clonal rearrangement could be detected								I	6

PI: Patient initials, **M:** Male, **F:** Female, **Dx:** Diagnosis, **TS:** Threshold sensitivity, **MR:** MRD-based risk group, **L:** Low, **I:** Intermediate, **H:** High, **NA:** Not available, **Neg.:** Negative, * Patients had either no clonal rearrangement or inadequate follow-up

The priority of our study was not only to investigate the clinical importance of MRD-based management of ALL but also to establish the laboratory procedures of molecular MRD monitoring for follow-up of patients receiving a standard chemotherapy.

Although time-consuming, laborious and costly, MRD monitoring is considered to be worthwhile, as it clearly indicates the relapse before it becomes clinically evident. Several studies reported that MRD positivity was associated with increased relapse rates. A greater proportion of these studies dealt with Ph chromosome-positive ALL patients, confirming that persistence or reappearance of bcr/abl positivity indicated a higher risk of relapse. Concordant results were obtained using immunoglobulin heavy chain (IgH)/TCR rearrangement as a marker for MRD in adult ALL. For example, semi-quantitative IgH gene analysis in a series of 85 adult precursor B-lineage ALL (B-ALL), done by Mortuza et al. [15] during the first 24 months of treatment, showed that MRD positivity was associated with increased relapse rates.

Although no statistical evaluation could be done due to our low patient number, our data are in line with the results of recently published studies confirming the evidence that the molecular MRD status of bone marrow is a strong predictor of outcome in adults with ALL. All of our patients with bad prognostic translocations died within a median of 14 months despite intensified chemotherapy for high-risk patients. Only patient MÇ, who became MRD-negative in the 3rd month (D90) and preserved MRD negativity for about six months, survived longer. Her disease relapsed soon after MRD was determined positive.

In line with the literature, MRD-based low- and intermediate-risk patients in our study had a significantly higher overall survival than high-risk patients (35 and 37 months versus 18 months) [20]. The slightly higher survival in the intermediate-risk group over the low-risk group (37 months versus 35 months) can be explained by the inclusion criteria for the MRD-based intermediate-risk group. This risk group, by definition, included all patients with inconclusive and/or technically incomplete MRD monitoring. A significant number of our intermediate-risk patients would have probably fallen into the low-risk category if they had had interpretable or technically available results.

In conclusion, MRD monitoring is progressively becoming a more important predictive factor in adult ALL patients. As reported by others and confirmed by our limited data, there is a good correlation between MRD status and clinical outcome in patients receiving chemotherapy. The guidance of MRD monitoring will result in MRD-based individualization of treatment protocols, leading to tailoring of unnecessary consolidations in some low-risk patients while alerting to the need for transplantation in others.

To keep pace with the evolving molecular biology and genetic techniques and current leukemia management strategies requiring central MRD monitoring, Turkey has to develop new policies and the necessary basic services. Having experienced the difficulties of establishing a “new” system, we think we have opened a door towards developing a central laboratory for MRD follow-up. We hope that our contribution will bring country-wide interested parties together to work on a large-scale MRD study in ALL.

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