Original Article

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The Evaluation of Multidrug Resistance-Related Protein 1 as a Prognostic Factor in the Pediatric B-cell Acute Lymphoblastic Leukemia: A Pilot Study

Pediyatrik B-hücreli Akut Lenfoblastik Lösemi Hastalığında Prognostik Faktör Olarak Multidrug Resistance-Related Protein 1 Değerlendirilmesi: Pilot Çalışma

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ABSTRACT

Objective: Acute lymphoblastic leukemia (ALL) is the most prevalent type of cancer in children. Minimal residual disease (MRD) is still the most important indicator of clinical results and relapse after chemotherapy. Multidrug resistance is the main obstacle to successful treatment. Multidrug resistance-related protein 1 (MRP1) may play a key role in throwing the chemical drug out of cells leading to therapy resistance. This study aims to detect MRP1 protein in the bone marrow cells of children with B-ALL and determine its value as a prognostic factor in comparison with other factors such as DNA index and MRD obtained by flow cytometric measurement.

Methods: Bone marrow samples were obtained from children who are diagnosed as B-ALL (n=20) at day 0 (diagnosis) and 15 of therapy. Risk groups' classification is based on discrimination of age and white cell count on day 0. The expressions of MRP1 levels and DNA index at diagnosis and MRD on the 15th day of treatment in the bone marrow were detected by using flow cytometry. The B-ALL blast cells were stained using anti-CD10, -CD19, -CD20, -CD34, -CD45 monoclonal antibodies. MRP1 content of cells was detected in an intracellular manner.

ÖZ

Amaç: Akut lenfoblastik lösemi (ALL), çocuklarda en sık görülen kanser türüdür. Kemoterapinin ardından, minimal kalıntı hastalık (MRD) hala klinik sonuçların ve nüksün en önemli göstergesidir. Çoklu ilaç direnci başarılı tedavinin önündeki ana engeldir. Çoklu ilaç direnci ile ilişkili protein 1 (MRP1) kimyasal ilacın hücrelerden dışarı atılmasında anahtar rol oynayabilir ve tedaviye dirence neden olabilir. Bu çalışmanın amacı, B-hücre ALL'li çocukların kemik iliğinden alınan hücrelerde MRP1 proteinini saptamak ve akan hücre ölçer ile elde edilmiş DNA indeksi ve MRD gibi diğer faktörlerle karşılaştırılarak MRP1'in prognostik bir faktör olarak değerini belirlemektir.

Yöntemler: B-ALL (n=20) tanılı çocuklardan tedavinin 0. (tanı anı) ve 15. gününde kemik iliği örnekleri alındı. Risk grupları tanı anındaki yaş ve beyaz hücre sayısına göre ayrılmıştır. Kemik iliğinde tanı anındaki MRP1 düzeyleri ve DNA indeksi ve tedavinin 15. gününde MRD düzeyi akan hücre ölçer kullanılarak tespit edildi. B-ALL blast hücreleri anti-CD10, -CD19, -CD20, -CD34, -CD45 monoklonal antikorlar kullanılarak boyanmıştır. Hücrelerinin MRP1 içeriği hücre içi boyama yöntemi ile belirlenmiştir.

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Results: There was no statistically significant difference in MRP1 expression between risk groups and the other prognostic factor as Flow MRD and DNA index.

Conclusion: The utilization of MRP1 as a predictive factor may not provide information on the B-ALL prognosis. Our results can help to better understand the nature of MRP1 in B-ALL patients.

Keywords: B-cell acute lymphoblastic leukemia, multidrug resistance-associated protein 1, multidrug resistance-related protein 1, minimal residual disease, DNA index, flow cytometry

Bulgular: Risk grupları ile diğer prognostik faktörler flow MRD ve DNA indeks arasında MRP1 ekspresyonu açısından istatistiksel olarak anlamlı bir fark yoktu.

Sonuç: MRP-1'in protein seviyeleri B-ALL prognozu hakkında bilgi sağlamayabilir. Sonuçlarımız, B-ALL hastalarında MRP-1'in doğasını daha iyi anlamaya yardımcı olabilir.

Anahtar Sözcükler: B-hücreli akut lenfoblastik lösemi, çoklu ilaç direnci ile ilişkili protein 1, multidrug resistance-related protein 1, minimal kalıntı hastalık, DNA indeksi, akan hücre ölçer

Introduction

Leukemia is made up of two Greek words of "*leukos*" and "*emia*" and refers to the term "white blood" and describes its characteristics as a cancer of the blood that appears with high numbers of malignant white blood cells (leukocytes) and it is the most common pediatric cancer and the cause of 29% of all cancer-related deaths in children between 1 and 14 years (1). The acute form of childhood leukemia is the most frequent (2). Acute lymphoblastic leukemia (ALL) is present in over 80% of children with ALL and B-cell ALL comes from an uncontrolled expansion of anomalous immature B-lymphocytes (3). ALL is one malignant disease most prevalent in childhood with the greatest therapeutic progress in the last three decades. This progress was achieved by recognizing certain prognostic risk factors which enabled the treatment to be adjusted accordingly. Many major risk factors are used in diagnosis and treatment stratification. These include age and white cell count (WCC), DNA index, as well as treatment response (4). According to National Cancer Institute (NCI) stratification, a worse prognosis is associated in patients with age ≥10 years and/or initial WCC of ≥50,000 per mm^3 (5). In addition, the DNA index is an independent prognostic factor and gives valuable information for diagnostic and treatment stratification. Studies have demonstrated that hyperdiploidy (DNA index >1.16) is associated with a better or favorable prognosis in ALL (6,7). As well as, minimal residual disease (MRD), assessed by molecular or flow cytometric manner, is considered the most important indicator of clinical results and relapse in childhood. Researchers have demonstrated that a high level of MRD is associated with an unfavorable prognosis regardless of the other risk markers commonly used for risk stratification (8).

Chemotherapy is the main way of treating leukemia. Drug resistance in patients diagnosed as having leukemia is often considered the principal clinical obstacle to efficient chemotherapy. Nowadays, several resistance mechanisms are identified, and the most important and common mechanism for chemotherapy failure is multidrug resistance (MDR) (9). Many MDR drug efflux pumps belong to the superfamily of ATP-binding cassette (ABC) transporters. Multidrug resistance-related protein 1 (MRP1) also belongs to the ABC transporter family. Findings indicate that MRP1 plays a key role in eliminating and sequestrating chemotherapeutic drugs, which results in reduced levels in concentrations at their target locations (10). However, the precise clinical value of these MDR proteins in ALL childhood is not clear and the data available are contradictory.

The purpose of this paper is to assess the importance of MRP1 as a predictor for responsiveness to therapy of childhood B-ALL. It is questionable whether the detection of MRP1 at diagnosis is an independent prognostic factor for B-ALL or not. Depending on certain actual independent predictive factors like DNA index at diagnosis and the MRD risk evaluation on the 15th day of treatment, the correlation of MRP1 expression with these factors was investigated.

Methods

Patients

Bone marrow samples obtained from patients with newly diagnosed and untreated ALL (n=20; 10 females, 10 males; age mean: 7.83; age range 3-15 years) from 2017 through 2018 were enrolled in the study after their parents signed the informed consent forms. The study was approved by the local ethics committee (17.10.2017/1143).

The bone marrow samples were collected in a tube with EDTA obtained from patients. The samples were used at the time of diagnosis, for immunophenotyping of leukemia, MRP1 expression of different cell subsets, and DNA index of B-ALL patients. On day 15th day of treatment, MRD levels were analyzed. All parameters were examined by the flow cytometric method.

Blast Characterization

The B-ALL panels were selected concerning over-expressed markers and for the B-ALL phenotype. Blasts were labeled by anti-CD10 phycoerythrin (PE) or PE-Cyanine 7 (Cy7), -CD19 allophycocyanin, -CD20 fluorescein isothiocyanate (FITC), -CD34 PE and -CD45 peridinin chlorophyll protein complex labeled monoclonal antibodies with "stain, lyse and then wash" approach (all monoclonal antibodies from BD Biosciences, USA). Samples were directly stained with a four-color monoclonal antibody cocktail at room temperature for 15 minutes in the dark. After incubation, 2 mL of FACS Lysing Solution (Becton Dickinson; BD Bioscience, San Jose, USA) was added and incubated for 10 minutes at room temperature in the dark. Following the lysis step, cells were washed with 2 mL phosphate-buffered saline (PBS), resuspended in 500 mL PBS and subsequently, data were acquired by flow cytometry

(FACSCalibur, Becton Dickenson, San Jose, USA) with CellQuest software (Becton Dickenson, San Jose, USA).

MRP1 Staining

Intracellular staining protocol was used for the detection of MRP1 expression of bone marrow cells. After labeling surface markers with anti-CD10, -CD19, -CD45 monoclonal antibodies, started intracellular staining through two steps: fixation and permeabilization (FIX & PERM, Nordic Mubio, Netherlands). In both stages, the cell suspension was incubated for 15 minutes in the dark followed by washing steps with PBS. The last incubation step was performed with permeabilization reagent and anti-MRP1 FITC antibody (BD Bioscience, San Jose, USA) together. The detection of MRP1 florescence bone marrow-derived cells: lymphoid, monocytes, myeloid cells, and blasts ($CD10^{+}CD19^{+}CD45^{neg/dim}$) was done on flow cytometry regarding fluorescence minus one control.

DNA Index

Estimation of the cellular DNA content was performed by flow cytometry. Mononuclear cells from bone marrow mononuclear cells were isolated by density gradient centrifugation using Ficoll-Paque (Histopaque-1077; Biochrom, Cambridge, UK) at 2,100 x rpm for 30 min. The buffy coat was washed twice with PBS, then stained by propidium iodide after digestion with enzymes trypsin and ribonuclease A using the BD Cycletest Plus DNA Reagent Kit (BD Cycletest, San Jose, USA) according to Vindelov (11). The DNA content was analyzed with a computer program Modfit LT by histograms. DNA index is defined as the ratio of the mean channel of G0/G1 cells of an aneuploid population divided by the normal diploid of G0/G1 mean channel (12).

Minimal Residual Disease

The bone marrow samples obtained from the patients on the 15th day of treatment were identified with specific monoclonal antibodies (CD10, CD11a, CD19, CD20, CD34, CD45, and CD58) according to the phenotypic characteristics and ALL IC-BFM 2009 Standard Operating Procedure. The residual blast cells were determined in $CD19^{+}$ gate by displaying lymphoid-scattering properties and leukemia-associated immunophenotypic characteristics as a percentage by flow cytometry (13).

Statistical analysis

In conjunction with the IBM SPSS Statistics for Windows, Version 21.0. (Armonk, NY: IBM Corp. USA) program, the data collection and management were performed using the Microsoft Office Excel package. Non-parametric methods such as Mann-Whitney U test were used and $p<0.05$ was considered statistically significant. The data that were continuous variables were presented as median (minimum-maximum).

Results

Clinical Features and Risk Group Stratification

The clinical and laboratory data of 20 patients with B-ALL are shown in Table 1. Patients were grouped according to standard-risk [(SR), n=8] and high-risk [(HR), n=12] ALL. This classification was based on discrimination of age and WCC. SR-ALL group characteristics were WCC less than 50,000/μL and age 1-9 years, and HR-ALL group characteristics were WCC ≥50,000/μL and/or age 10 years or older (5).

DNA index was measured as 1.15 (1-1.83) in 20 patients and it was ranging from 1 to 1.15 in 12 (60%) patients as hypodiploidy and ranging from 1.17 to 1.83 in 8 (40%) patients as hyperdiploidy (7). All HR patients had DNA index <1.16 and all SR patients were hyperdiploid (DNA index >1.16). According to this correlation, study groups were identified as SR and HR (Table 2).

MRP1 Expression of Bone Marrows Cells of SR and HR Risk Group B-ALL Patients

The expression MRP1 was investigated in 20 patients with B-ALL and determined by flow cytometry by direct immunofluorescence intracellular staining. MRP1 positive cells were determined by reference to isotype control (BD Bioscience, San Jose, USA). Lymphocytes, monocytes, and myeloid cells were gated on SSC/CD45 dot plot (Figure 1). Blast cells were gated on CD10/CD45 dot plot obtained from CD19 positive cells (Figure 2). There was no statistical difference between SR and HR risk groups in terms of bone marrow cells ($p>0.05$) (Figure 3).

Day 15 Minimal Residual Disease and MRP1 Expression

On the 15th day of therapy, the bone marrow samples obtained from patients with B-ALL were examined for MRD by flow cytometry. The percentages of leukemic cells were classified

Table 1. Clinical characteristics of patients with B-ALL

Demographic data [median (minimum-maximum)]		
Number of patients	20	
Age (year)	4.5 (3-15)	
Sex (male/female)	10/10	
Clinical features		**Normal ranges**
WCC ($x10^9$/L)	68.4 (12.4-186.4)	5.0-17.0
RCC ($x10^{12}$/L)	3.2 (0.1-3.9)	3.9-5.3
Hemoglobin (g/dL)	9.7 (0.3-13.0)	115-135
Platelet count ($x10^3$/uL)	339.0 (42.0-876.0)	150-400
Blast cells (%)	82.2 (63.9-95.3)	0.0-2.0

WCC: White cell count, RCC: Red cell count

Table 2. Distribution of patients with B-ALLaccording to DNA index and risk (based on age and WCC): High and standard risk groups were associated with hypodiploidy and hyperdiploidy groups, respectively

DNA index	Risk stratification		
	HR	SR	N
Hypodiploidy (<1.16)	12	0	12 (60%)
Hyperdiploidy (>1.16)	0	8	8 (40%)
Total	12 (7F/5M)	8 (3F/5M)	20 (10F/10M)

F: Female, M: Male

as flow low risk (FLR) with blast cells <0.1%, flow medium risk (FMR) with blast cells between 0.1-10%, and flow high risk (FHR) with blast cells >10% (13). One sample with a normoblast rate of less than 2% (1.6%) was excluded because of hemodilution. There were two patients with FHR, 16 patients with FMR, and one with a negative result that was considered as FLR. One patient who had negative MRD and FLR was HR at diagnosis and two patients who had weak early response to therapy had FHR and MRD >10% (15.9297 and 66.6032% MRD) and were SR at diagnosis. The majority of our patients (85%) had a medium response (FMR) (Table 3). Patients with FHR and FLR were excluded because of their low numbers. MRP1 expression of bone marrow cells of FMR-SR (n=5) and FMR-HR (n=11) groups were compared and there was no difference (p>0.05, Figure 3). Furthermore, patients with B-ALL with FMR were ranked depending on the CD34 phenotype of blasts as FMR-34 negative (n=3) and positive (n=13) (Table 3).

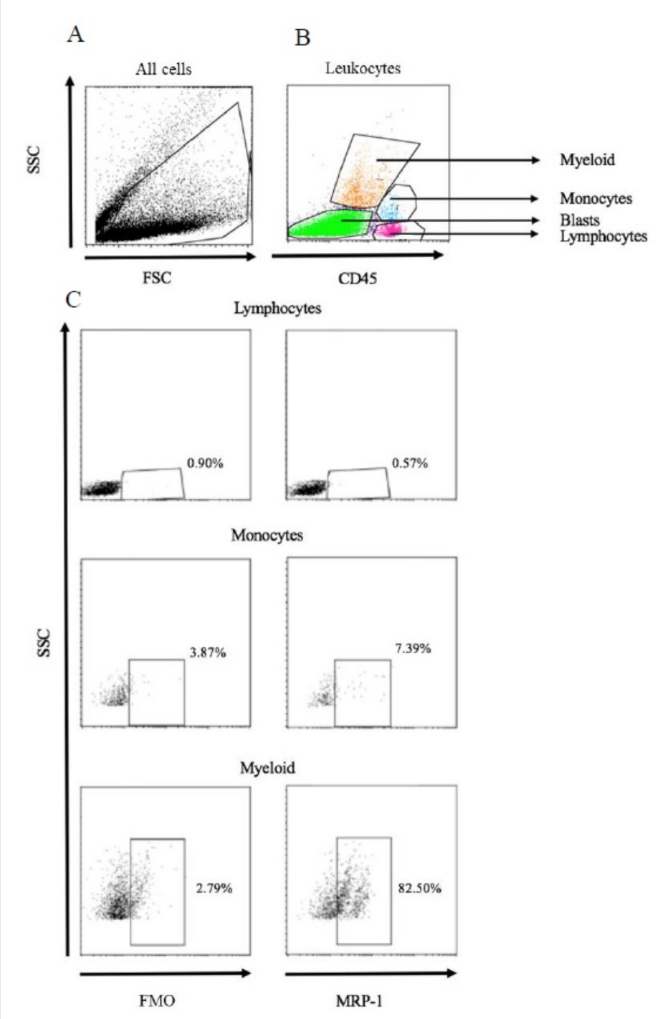


Figure 1. Gating strategy for detection of MRP1 in different bone marrow-derived cells: lymphocytes, monocytes, and myeloid cells. Leukocytes were gated on SSC versus FCS graph (A), the subdivision of bone marrow cells discriminated by their CD45 expression intensity (B), MRP1 expression of each subpopulation was determined regarding FMO (C). FMO: Fluorescence minus one control

Two subgroups were compared in terms of MRP1 expressions and there was no difference between them (p>0.05, Figure 3).

Discussion

MDR which is due to the overexpression of many efflux proteins such as MRP1 can traffic chemotherapeutic drugs from the cell. Although the role is not fully understood, the importance and the influence of MRPs on the strategy and outcome of clinical cancer treatment cannot be neglected (14). It has been suggested that nuclear MRP1 can be a better prognostic factor in human mucoepidermoid carcinoma (15). But, in breast tumor tissues, low level of MRP1 is considered as a predictive biomarker for the patients responding to chemotherapy (16). An investigation proposed an alternative pathway to understanding the failure of therapy after assessment of MRP1 content by quantitative polymerase chain reaction (PCR) in lymph node biopsy

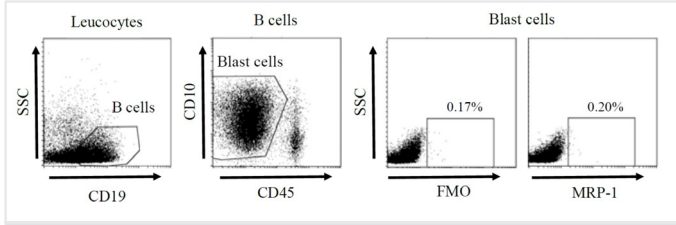


Figure 2. Gating strategy of MRP1 expression in blast cells. CD19 positive B cells were extracted from leukocytes and gated on SSC versus CD19 dot plot (A). B cells with CD10+CD45neg/dim phenotype were gated on CD10 versus CD45 scatter graph as Blast cells (B). And finally, blast cells' MRP1 expression was determined by SSC versus MRP1 regarding FMO. FMO: fluorescence minus one

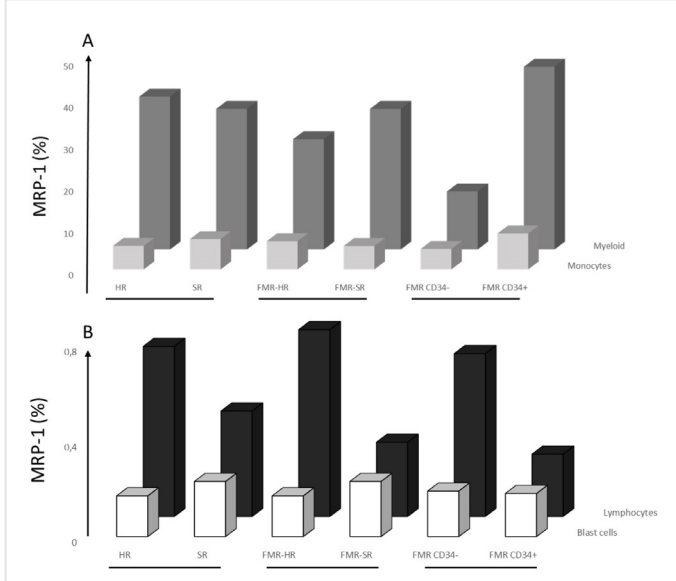


Figure 3. MRP1 expression (median %) of bone marrow cells of B-ALL patient HR (n=12) and SR (n=8), FMR-HR (n=11) and FMR-SR (n=5), FMR with (n=13) or without (n=3) CD34 blast phenotype expression. (A) Monocytes, myeloid cells, (B) blast cells, and lymphocytes

specimens from a group of patients with lymphoma (non-Hodgkin's lymphoma, non-malignant lymphadenopathy, and Hodgkin's lymphoma) (17). Many studies investigated expression levels of MRP1 mRNA and showed poor prognosis related to childhood ALL and reported significantly higher expression at relapse in association with age >15 years or age-independent or with poor 2-year survival (10,18-22). As concerning resistance to chemotherapy, MRP1 was suggested as one of the mechanisms responsible for induction failure, also in adult patients with ALL (23).

Many studies have not confirmed the correlation between overexpression of MRP1 and clinical drug resistance in hematologic malignancies. The expression of MRP1 mRNA shown by PCR appeared to have no prognostic importance and no association with risk factors such as phenotype, age, and leukocyte count in newly diagnosed patients with childhood ALL and it was not related to a worsening of event-free survival (24,25). It was also shown that the expression of Pgp, MRP1, and LRP was not related to WCC and age, and did not contribute to treatment failure (26-28). Also, MRP1 measurement at the protein level by FCM analysis appeared to have no concordance with the clinical aspect (29). In an *in vitro* study, cytotoxicity of 4-hydroxy-ifosfamide, daunorubicin, and prednisolone on peripheral blood lymphocytes was assessed by the MTT assay and a weak correlation was found with high MRP protein expression which suggested that MRP was unlikely to be involved in drug resistance (30).

MRD is a demonstration of drug resistance and is correlated significantly with the NCI risk group. HR patients are significantly more likely to be MRD-positive than SR patients (31). The DNA index measurement is important for treatment stratification. In the earlier studies it was shown that SR patients were associated with a DNA index greater than 1.16 (hyperdiploidy) and that it was a favorable factor such as age between one and ten years and WCC lower than $50x10^9$/L (7,32). We examined the baseline WCC, age and DNA index, and found that all HR patients were simultaneously hypodiploid and SR patients were hyperdiploid matching

Table 3. General aspects of patients with B-ALL in terms of risk stratification (regarding age/WCC, DNA index, and flow MRD), phenotype (CD10, CD20, CD34, and CD45 expressions), and MRP1 expression in bone marrow cells

Patient No	Age (year)	Sex	Bone marrow WCC ($x10^9$/L)	Risk	DNA index	CD10 (%)	CD19 (%)	CD20 (%)	CD34 (%)	CD45 (%)	MRP-1 (%) in gate: Blast	Lymphoid	Monocytoid	Myeloid	Norm (%)	MRD (%)	MRD risk
1	3	F	69.43	HR	1.07	98.48	98.57	5.37	73.60	49.42	0.17	0.26	10.97	46.65	3.86	0.9326	FMR
2	3	F	121.60	HR	1.08	97.53	97.54	94.78	97.70	6.64	0.45	1.67	1.97	17.95	2.52	0.1071	FMR
3	6	F	186.36	HR	1.09	98.45	98.49	1.39	0.49	97.74	0.12	1.59	3.01	6.31	18.44	0.8678	FMR
4	5	M	24.61	SR	1.19	75.89	85.24	2.83	0.44	28.19	0.20	0.25	1.19	2.79	3.10	1.5732	FMR
5	7	F	32.99	SR	1.83	5.83	95.05	0.93	2.81	100	0.15	0.50	15.38	1.51	8.20	15.9297	FHR
6	9	F	81.34	HR	1.12	42.52	96.88	30.83	42.14	99.51	0.19	1.82	6.73	6.28	2.64	4.3599	FMR
7	3	M	74.25	HR	1.15	97.70	98.11	3.85	84.82	65.05	0.17	0.22	4.29	65.31	58.00	0.0000	FLR
8	12	F	110.59	HR	1.15	81.84	99.50	6.00	94.44	99.66	0.18	0.91	8.57	63.46	61.10	6.8486	FMR
9	2	M	67.35	HR	1.15	98.64	98.66	26.58	98.53	3.75	0.14	0.64	2.69	8.81	9.16	0.1391	FMR
10	5	F	30.31	SR	1.23	93.26	93.59	38.80	62.26	26.16	0.25	0.67	8.88	58.51	4.80	66.6032	FHR
11	4	M	12.47	SR	1.17	70.89	98.46	10.51	74.10	70.58	0.24	0.24	9.80	43.56	14.84	0.7524	FMR
12	10	M	115.90	HR	1.11	98.96	99.21	3.41	3.51	80.02	0.12	0.60	16.15	89.33	19.74	1.4366	FMR
13	15	F	29.25	HR	1.14	85.88	98.10	4.28	98.86	68.8	0.23	1.45	14.46	55.69	19.50	9.2981	FMR
14	13	M	85.61	HR	1.13	7.11	97.95	2.01	98.05	97.8	0.09	0.10	1.11	1.77	18.40	7.547	FMR
15	3	M	30.45	SR	1.35	98.22	98.24	35.59	29.60	26.7	0.28	0.73	7.03	89.76	31.90	0.7942	FMR
16	4	F	26.45	SR	1.25	89.87	89.98	74.40	86.26	41.83	0.26	0.57	7.39	82.50	32.01	1.6212	FMR
17	6	M	28.05	SR	1.42	98.24	98.35	1.99	95.42	6.14	0.15	0.22	3.06	11.77	1.65	6.7534	FMR
18	3	F	132.88	HR	1.00	94.60	94.73	1.84	60.86	2.32	0.17	0.26	4.50	26.30	4.44	0.7064	FMR
19	3	M	40.36	SR	1.28	93.23	93.59	3.69	68.07	86.32	0.22	0.38	4.13	23.52	4.68	0.3396	FMR
20	3	M	99.46	HR	1.08	72.69	99.49	3.02	92.60	61.82	0.46	0.78	62.56	66.03	19.00	9.2005	FMR

	Age	Sex	Bone marrow WCC ($x10^9$/L)	Risk	DNA index	CD10, CD19, CD20, CD34, CD45 (%)	MRP-1	Normoblast	MRD Risk
	>10 years	Male	>50	HR	Hypodiploidy	100-80	>2 SD	Adequate	FHR
						80-60	>1 SD		
						60-40	Mean		FMR
						40-20	<1 SD		
	<10 years	Female	<50	SR	Hyperdiploidy	20-0	<2 SD	Hemodilution	FLR

F: Female, M: Male, HR: High risk, SR: Standard risk, FMR: Flow medium risk, FLR: Flow low risk, FHR: Flow high risk, SD: Standard deviation, WCC: White cell count

Lustosa de Sousaa et al's study (32). And we observed no relation with risk stratification criteria regarding WCC/age/DNA index and, MRP1 of which expression was examined by flow cytometry at the time of diagnosis of newly diagnosed patients. We evaluated the cell types of the bone marrow according to CD45 fluorescence characteristics as lymphoid, monocytes, myeloid, and $CD10^+CD19^+$ blast cells, and their MRP1 levels. On the 15th day of therapy which was administered according to initial risk, the response was determined. MRD in bone marrow was examined by flow cytometry and 85% of our patients had FMR. The number of patients with FLR (n=1) and FHR (n=2) were too small to compare, so only patients with FMR response were grouped according to WCC/age/DNA index risk at the time of diagnosis and MRP1 protein levels were compared. Thus, in our study, no difference was found in terms of baseline MRP1 expression of HR and SR patients who had FMR response on day 15th. Patients with relapse were not included in this study.

Drach et al. (33) and Chauhan et al. (23) showed the association of the MDR-1 gene expression with immature immunophenotype as $CD34^+$ cell lineage. On the other hand, there was an observation of the similarity of MRP gene expression levels in AML and normal $CD34^-$ and $CD34^+$ bone marrow cells (18). We found high MRP1 protein expression in myeloid cells of bone marrow samples from patients with B-ALL , but there was no significant difference between risk groups. Also, our findings did not determine the relation of MRP1 expression in blast cells with phenotype either $CD34^-$ or $CD34^+$.

From point of view of gender, our finding conflicted with an earlier study that considered male gender as an independent prognostic factor and associated it with poor prognosis (34). The number of male children in this study was equal to the number of female children. In the examination of the distribution of gender to WCC/age/DNA index, male subjects showed equal distribution and the majority (70%) of female subjects were in the HR group. We could not prove strongly that the male children had a poor prognosis because of their low number.

Study Limitations

Some established poor genetic factors such as IKZF1 deletion, KMT2A rearrangements, and the Philadelphia chromosome are considered molecular indicators of high risk for failure of treatment or resistance to treatment. Because of scarce data about the presence of this factor, we couldn't evaluate the relationship with MRP-1.

Conclusion

In this study, the expression of MRP1 protein at diagnosis whether to help or not as a predictive factor for prognosis of patients with B-ALL was investigated. Although the low number of patients made it difficult to compare subgroups, our study focused on the level of MRP1 protein expression in different bone marrow cell subsets by comparing risk factors: WCC, age, DNA index, and CD34 positive/negative phenotype groups regarding their therapy response. We think that the cause of conflicting results in the literature is the disease phenotype ($CD34^-$ and $CD34^+$) or cell subgroups (lymphocytes, monocytes, myeloid and blast cells) cannot be evaluated separately. Measuring MRP1 by flow cytometry is advantageous than western blot and PCR because it is easily analyzed in individual different cell groups including blast cells, although intracellular staining has disadvantages as fixation and permeabilization procedures that can damage cell integrity. In conclusion, our findings do not support MRP1 protein expression as a prognostic factor and as a predictor for drug resistance.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee (17.10.2017/1143).

Informed Consent: The study after their parents signed the informed consent forms.

Peer-review: Externally peer reviewed.

Authorship Contributions

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