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MONITORING OF MINIMAL RESIDUAL DISEASE IN BONE MARROW AND PERIPHERAL BLOOD IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

PRAĆENJE MINIMALNE REZIDUALNE BOLESTI U KOSTNOJ SRŽI I PERIFERNOJ KRVI KOD PACIJENATA SA AKUTNOM LIMFOBLASTOM LEUKEMIJOM

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Ključne reči
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kostna srž (BM)

Abstract

Modern treatment protocols for acute lymphoblastic leukemia (ALL) include the analysis of minimal residual disease (MRD), the detection of small number of malignant cells that survived after therapy. MRD diagnostics represents the most sensitive method to evaluate treatment response and the strongest prognostic factor in ALL patients. Detection of MRD is commonly based on polymerase chain reaction (PCR) analysis of rearranged immunoglobuline heavy chain (IGH) or T-cell receptor (TCR) genes in bone marrow (BM) samples. Since BM sampling is invasive and traumatic, the aim of this study was to evaluate clinical usefulness of MRD monitoring in the peripheral blood (PB). The study included 59 patients with ALL (44 B-ALL and 15 T- ALL). Samples of PB and BM were taken after induction therapy and during the follow-up. Clonality testing was based on PCR analyses of rearranged IGH or TCR gamma genes. In patients with B-ALL, MRD was detected in BM of 10 patients and in PB of 6 patients. In T-ALL, MRD was detected in BM of 7 patients and in PB of 6 patients. Obtained results indicated that in patients with B-ALL MRD assessment of BM is more informative than analysis of PB, while in T-ALL traumatic BM sampling might be replaced with PB.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant disorder characterized by the overproduction and accumulation of cancerous immature white blood cells (lymphoblasts). Conventional diagnostic and prognostic criteria are based on clinical, laboratory, immunophenotypic and cytogenetic parameters [1, 2]. A majority of adult patients with newly diagnosed ALL achieve a morphologic and cytogenetic complete remission and up to one-half of them experience prolonged disease free survival (DFS) [3]. However, despite achieving morphological remission, there are a significant number of patients who relapsed. The relapse is the result of the presence of residual leukemic cells that are undetectable by standard morphologic and cytogenetic analyses. The presence of leukemic cells detectable only with sensitive molecular analyses is designated as minimal residual disease (MRD) [4]. The MRD status in both childhood and adult ALL, significantly correlates with clinical outcome[5].

In the early 1980s classical Southern blotting of rearranged immunoglobulin (IG) and T-cell receptor (TCR) genes and flow cytometry were used for detection of MRD [5]. These methods detect malignant population if it makes at least 5-10% of total lymphocyte population which appeared to be not enough sensitive for the MRD detection. The introduction of PCR into clinical practice as well as improvement of flow cytometry brought higher sensitivity in MRD analyses (10^{-4} - 10^{-5}) [6]. Presently, recommended methods for MRD detection include PCR or RQ-PCR analyses of specific IG/TCR genes rearrangements or specific genetic aberrations [7, 8].

Over the last decade MRD diagnostic has proven to be the strongest prognostic factor in patients with ALL. The presence/quantity of MRD at the completion of remission induction therapy divides ALL patients into different risk groups, ranging from low-risk/standard risk to medium-risk or high-risk [7, 9].

MRD analyses at the completion of induction therapy are commonly performed on bone marrow (BM) samples (10). Several studies suggested that peripheral blood samples might be used instead of invasive and traumatic BM samples, particularly in T-ALL derived from progenitor cells that naturally reside in the thymus and migrate to the BM [10, 11]. The aim of this study was to evaluate clinical usefulness of MRD monitoring in the PB.

MATERIAL AND METHODS

Patients and samples

A total of 59 ALL patients were included in the study: 44 B-cell ALL and 15 T-cell ALL. Patients were diagnosed and treated at the Clinic for Hematology of Military Medical Academy, Belgrade. All patients were diagnosed according to standard criteria and classification [12]. Samples of PB and BM were taken during the follow-up period (one month after induction therapy and subsequently in three- or six-months intervals, depending on the course of disease).

DNA was extracted from PB/BM mononuclear cells by *Quick*-gDNA™ Miniprep Kit (ZYMO RESEARCH, The Epigenetics COMPANY™, USA). Clonality testing was based on PCR analyses of rearranged IGH or TCR gamma genes. PCR protocols for B- and T-cell clonality assesment were described previously [13]. PCR products were considered to be monoclonal (MRD-positive) if only one discrete band within the expected size range of 100-150 bp for B-cell clonality, or ~ 200 bp for T-cell clonality, was observed on the gel after electophoresis. Polyclonal PCR products were considered as MRD-negative result (figure 1).

RESULTS

MRD in B-ALL

The present study included 44 patients with B-ALL. B-cell clonality testing was performed in PB and BM samples during the follow-up period.

MRD was detected in BM of 10 patients and in PB of six patients (p=0.408, Fisher test). The presence of MRD in both, BM and PB was detected in four patients, six patients had MRD only in BM, while two patients had residual leucemic cells only in PB.

The presence of MRD in BM and/or PB samples of B-ALL patients is presented in table 1.

MRD in T-ALL

15 patients with T-ALL were included in the study. During the follow-up, MRD was detected in BM of 7 patients and in PB of 6 patients (p=1, Fisher test).

The presence of MRD in BM and/or PB samples of T-ALL patients is presented in table 1.

***Table 1**. Detection of minimal residual disease (MRD) in bone maroww (BM) and peripheral blood (PB) samples of patients with acute lymphoblastic leukemia (ALL).*

SAMPLE	B – ALL	T – ALL
BM+/PB+	4	6
BM+/PB-	6	1
BM-/PB+	2	0
BM-/PB-	32	8

\+ minimal residual disease is present in the sample;
\- minimal residual disease is not present in the sample.

DISCUSSION

A significant proportion of patients with ALL who achieve a clinical and morphological remission after induction therapy will relapse unless if additionaly treated with a kind of consolidation or intensification therapy. Previous reports have demonstrated that morphologically normal BM

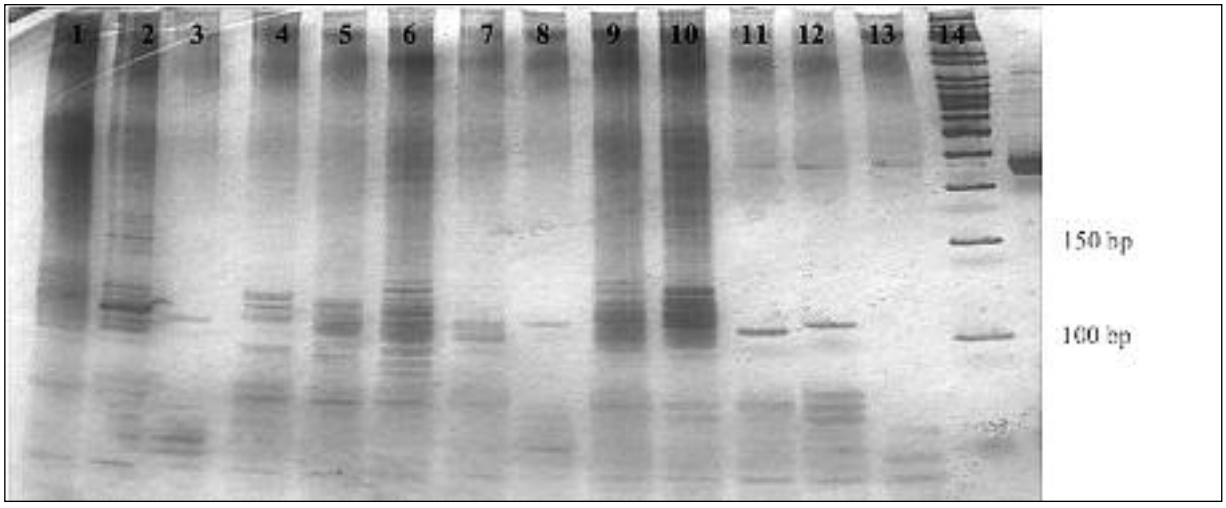

***Figure 1.** Detection of MRD using PCR-PAG method*
The presence of MRD was detected in lines 3,8 11 and 12.
Molecular weight marker was in the lane 14.

and PB are compatible with significant amounts of residual disease[14]. In the near past, a limited number of study groups in Europe and United States applied MRD diagnostic in clinical decission making for treatment of ALL. Presently, monitoring of MRD to evaluate treatment effectivness and MRD-based risk from relapse becomes routine practice in virtually all pediatric ALL and in majority of adult ALL cases in Western countries [7]. MRD assesment at the completion of induction therapy is routinely performed on BM samples. However, the BM aspiration is invasive and traumatic. From that reason, some authors suggested that PB might be used for MRD testing [10, 15].

Previous reports suggested that in T-ALL MRD analysis of PB might be as informative as analysis of BM since progenitor cells of T-ALL naturally residue in the thymus and migrate to the BM through the circulating blood [10, 11]). In the present study significant difference in MRD-positivity between BM and PB was not observed (p=1, Fisher test). Similar findings were reported by other study groups. In the study of Coustan-Smith and coworkers patients with T-ALL was positive for MRD in PB in all time points at which they were MRD-positive in BM. In addition, levels of MRD were remarkably similar in PB and BM samples (10). In the study of van der Velden and coworkers the MRD levels measured by real-time quantitative PCR in the paired BM and PB samples of patients with T-ALL were very comparable and strongly correlated. The same correlation between MRD levels in paired BM and PB samples was also obtained when immunophenotyping was used [15].

Considering B-ALL (progenitor cells residue BM), previous reports suggested that the presence of lymphoblasts in PB at the time of clinical remission indicate a propensity of malignant cells to leave the BM and invade extramedulary sites. The feature is usually associated with and poor outcome [11]. Our study included 44 patients with B-ALL; ten of them were MRD-positive in BM and six had MRD in PB. In four patients MRD was detected in both, PB and BM in all time-points during follow-up. Previous studied on B-ALL patients indicated that MRD analyses were more informative when performed in BM. For example, Coustan-Smith and coworkers found that patients with B-ALL often had no detectable leucemic cells in PB, despite their presence in BM [10]. In the study of van der Velden and coworkers MRD was detected in 107 BM-PB pairs: MRD-positivity was detected in both BM and PB (48 pairs), in 47 pairs solely BM was MRD-positive (at variable levels), and in 12 pairs only the PB sample was MRD-positive at very low levels. In double-positive pairs, MRD levels in BM and PB varied enormously (MRD levels in BM were up to 1000 times higher than in the corresponding PB samples)[15]. The study of Volejnikova and coworkers confirmed poor correlation between MRD in PB and BM of B-ALL patients, with levels in BM being higher than in BM in most samples [16].

The presence of MRD in PB, but not in the BM was found in two patients with B-ALL in our study and in 12 patients in the study of van der Velden and coworkers [7]. Possible explanation for false-negative BM samples might be found in the distribution of leucemic cells over BM. It has been speculated that ALL is relatively homogenously distributed over BM at diagnosis but that treatment might cause "patchy" distribution of leukemic cells, which might result in different MRD levels in different BM aspirates during follow-up [7]. However, some authors reported no signs for unequal distribution of ALL cells during treatment [17].

In summary, our study included qualitative, but not quantitative assessment of MRD in BM and PB. Obtained results indicated that in patients with B-ALL MRD assessment of BM is more informative than analysis of PB. However, in T-ALL traumatic BM sampling might be replaced with PB. Working with specific primers can increase the sensitivity of detection.

Sažetak

Savremeni protokoli za lečenje akutne limfoblastne leukemije (ALL) uključuju analizu minimalne rezidualne bolesti (MRD), otkrivanje malih količina malignih ćelija koje su preživele posle terapije. MRD dijagnostika predstavlja najosetljiviji metod za procenu odgovora na lečenje i najjači prognostički faktor kod ALL bolesnika. Detekcija MRD-a se najčešće zasniva na PCR analizi rearanžmana gena imunoglobulinskog teškog lanca (IGH) ili T-ćelijskog receptora (TCR) u uzorcima kostne srži (BM). Budući da je uzorkovanje BM invazivno i traumatično, cilj ove studije bio je procena kliničke korisnosti MRD monitoringa u perifernoj krvi (PB). Studija je obuhvatila 59 pacijenata sa ALL (44 B-ALL i 15 T-ALL). Uzorci PB i BM su uzeti nakon indukcione terapije i tokom praćenja. Testiranje klonalnosti je zasnovano na PCR analizama rearanžiranih IGH ili TCR gama gena. Kod pacijenata sa B-ALL, MRD je detektovana u BM kod 10 pacijenata i u PB kod 6 pacijenata. Kod T-ALL, MRD je detektovana u BM kod 7 pacijenata, a u PB kod 6 pacijenata. Dobijeni rezultati pokazuju da je kod pacijenata sa B-ALL procena MRD u BM više informativna nego analiza PB, dok se kod T-ALL traumatično uzorkovanje iz BM može zameniti analizom PB.

REFERENCES

1. Onciu M. Acute Lymphoblastic Leukemia. Hematol.Oncol.Clin.N Am. 2009; 23: 655-674

2. Pui CH, Robison L, Look AT. Acute lymphoblastic leukemia. Lancet . 2008; 371:1030-4

3. Brüggemann M, Raff T, Kneba M. Has MRD monitoring superseded other prognostic factors in adult ALL? Blood. 2012; 120(23):4470-4481

4. Parikh SK, Uparkar UP. Assessment of minimal residual disease in childhood acute lymphoblastic leukemia. J Appl Hematol. 2016;7:47-53

5. Campana D, Pui CH. Detectuon of minimal residual disease in acute leukemia: methodologic advances and clinical significance. Blood. 1995; 85: 1416-1434

6. van der Velden VH, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, et al. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR; principles, approaches, and laboratory aspects. Leukemia. 2003; 17:1013-34

7. van Dongen JJ, van der Velden VH, Brüggemann M and Orfao A Minimal residual disease diagnostics in acute lymphoblasic leukemia: need for sensitive, fast and standardized technologies. Blood. 2015; 125: 3996-4009

8. van der Velden VH, Cazzaniga G, Schrauder A, Hancock J, Bader P, et al. Analysis of minimal residual disease by Ig/TCR gene rearrangments: guidelines for interpretation of real-time quantitative PCR data. Leukemia. 2007; 26:604-611

9. van Dongen JJ, Seriu T, Panzer-Grümayer ER, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukemia in childhood. Lancet. 1998; 352(9142):1731-1738

10. Coustan-Smith E, Sancho J, Hancock LM, et al. Use of peripheral blood insted of bone marrow to monitor residual disease in children with acute lymphoblastic leukemia. Blood. 2002;100:2399-2402

11. Greaves MF. Differentiantion-linked leukemogenesis in lymphocytes. Science 1986;234:697-704

12. Harris NL, Jaffe ES, Diebold J, et al. 1999 World Health Organisacion clasification of neoplastic diesese of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting – Airlie House, Virginia, November 1997. J Clin Oncol. 1999;17:3835-3849

13. Cikota MB, Tukić JLJ, Tarabar TO, Stamatović TD Elez NM, Magić MZ. PCR-based clonality assessment in patients with lymphocytic leukemias: a single-institution experience. J Genet. 2009;88:309-314

14. Paietta E. Assessening minimal residual disease (MRD) in leukemia: a changing definition and concept? Bone Marrow Transplantation. 2002; 29(6):459-456

15. van der Velden VH, Jacobs DC,Wijkhuijs AJ et al. Minimal residual disease levels in bone marrow and peripheral blood are comparable in children with T cell acute lymphoblastic leukemia (ALL), but not in precursor-B-ALL. Leukemia. 2002; 16(8): 1432-1436.

16. Volejnikova J, Mejstrikova E, Valova T, Reznickova L, Hodonska L, et al. Minimal residual disease in pripheral blood at day 15 identifies a subgroup of childhood B-cell precursor acute lymphoblastic leukemia with superior prognosis. Haematologica. 2011; 96(12):1815-1821

17. van der Velden VH, de Bie M, van Wering ER, van Dongen JJ. Immunoglobulin light chain gene rearrangements in precursor-B-acute lymphoblastic leukemia:characteristics and applicability for detection on minimal residual disease. Haematologica. 2006; 91(5): 679-682

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