Exp Oncol 2008
30, 3, 248–252

Experimental ONCOLOGY

EXPRESSION OF *MDR1, LRP, BCRP* AND *BCL-2* GENES AT DIAGNOSIS OF CHILDHOOD ALL: COMPARISON WITH MRD STATUS AFTER INDUCTION THERAPY

U.U. Fedasenka, T.V. Shman, V.P. Savitski, M.V. Belevcev*
Belarusian Research Center for Pediatric Oncology and Hematology, 223053 Minsk, Belarus

Aim: **to investigate properties of leukemic cells by sorting out children diagnosed with ALL with different response to chemotherapy based on MRD level.** ***Methods:*** **We used a minimal residual disease (MRD) data on day 36 obtained with 3-colour flow cytometry as a reference. In view of MRD results, we used real-time PCR to assess expression levels of multidrug resistance associated genes *MDR1, LRP* and *BCRP*, antiapoptotic gene *Bcl-2* in initial samples from children diagnosed with ALL. P-gp expression and function in initial samples were analyzed by flow cytometry.** ***Results:*** **Briefly, medians of relative expression levels of *MDR1* gene were roughly comparable and in MRD^+ group came to 22.8 (0.02–26.6; n = 9) *vs* 24.8 (3.9–41.4; n = 10) in MRD^- group. *Bcl-2* gene showed tendency to higher expression levels in MRD^+ group with median at 5992.9 (521.0–10362.0; n = 9) compared to 3183.6 (1947.9–6581.0; n = 10) in MRD^- group. *LRP* gene relative expression levels were similar in both groups and came to 1934.9 (1500.7–3490.4; n = 9) and 1408.5 (665.5–2917.1; n = 10) in MRD^+ and MRD^- groups, respectively. The median of *BCRP* expression levels in MRD^+ group was considerably lower than that in MRD^- group, namely 76000.0 (48196.2–169230.8; n = 9) and 227967.2 (16683.7–422222.2; n = 10), respectively, but statistical analysis showed no significant difference for this parameter.** ***Conclusion:*** **We investigated expression of multidrug resistance genes *MDR1, LRP* and *BCRP* and antiapoptotic gene *Bcl-2* in leukemic cells at diagnosis, and MRD level at the end of induction therapy, and could not find obvious relations between these parameters.**
Key Words: **ALL, MRD, Bcl-2, MDR1, LRP, BCRP.**

Biological properties of the leukemic cells that survive chemotherapy have attracted attention of scientists for many years but still remain obscure. Although chemotherapy has markedly evolved and current risk adapted protocols succeed for majority of children diagnosed with acute lymphoblastic leukemia, approximately 25% of patients develop a relapse. A relapse is also an adverse prognostic factor and requires a high risk adapted treatment and consequently lead to longer hospitalization and increased drug toxicity [1].

Currently, multidrug resistance is often associated with an efflux of cytotoxic compounds by transmembrane proteins. Most of them belong to ATP-binding cassette superfamily transporters (ABC transporters) [2, 3]. Role of *MDR1* gene and its product P-glycoprotein in acute leukemia has been studied extensively by many investigators. Some of the relevant publications confirmed an association between complete remission rates, overall and relapse-free survival and *MDR1* gene or Pgp expression and/or function in acute leukemia [4–10]. Other studies failed to confirm these results [11–14]. Another ABC-transporter: breast cancer resistance protein (BCRP) and the corresponding gene also may play a role in acute leukemia but larger studies are needed to confirm this [10, 13, 15, 16]. Major vault protein or lung resistance protein (LRP) is often associated with multidrug resistance [11, 17, 18]. This protein is a part of the vault complex, a eukaryotic barrel shaped organelle with a recently described structure but still unknown cell function. Current data on partial colocalization of vaults with cytoskeletal elements, secretory organelles and nucleus suggests its function as a transporter [19–23]. Several studies have already demonstrated a negative role of LRP expression in prognosis of acute leukemia [11, 17, 18, 24–26,]. In contrast, other investigators did not point towards a prognostic significance of LRP [12, 27–29].

Surviving during chemotherapy also depends on ability of cells to undergo apoptosis. Antiapoptotic gene/protein Bcl-2 is known to be associated with response and remission rate in acute leukemia, although there were publications with controversial data [30, 31].

In our study we attempted to investigate properties of leukemic cells by sorting out patients with different response to chemotherapy. We used a minimal residual disease (MRD) data on day 36 obtained with 3-colour flow cytometry for ALL patients as a reference. In view of MRD results, we assessed expression levels of multidrug resistance associated genes *MDR1, LRP* and *BCRP*, antiapoptotic gene *Bcl-2* and P-glycoprotein expression and function in initial samples from children diagnosed with ALL.

METHODS

Patients. 19 children diagnosed with primary B-lineage ALL were enrolled into the study. The patients age ranged from 1,9 to 18 years with median age at 5,5 years. The study was approved by the Ethical committee of the Research Center, samples were obtained under consideration of all legal requirements.

Received: July 7, 2008.
*Correspondence: Fax: 375 (0)17 265 42 22
E-mail: januarys_v@yahoo.com

Abbreviations used: ABC – adenosine tri-phosphate-binding cassette; ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia; Bcl-2 – B-cell chronic lymphocytic leukemia/lymphoma 2; BCRP – breast cancer resistance protein; cDNA – complementary deoxyribonucleic acid; Cy5 – cyanine; FACS – fluorescent activated cell sorter; FITC – fluorescein isothiocyanate; MAb – monoclonal antibody; MDR – multidrug resistance; MRD – minimal residual disease; LRP – lung resistance protein; PCR – polymerase chain reaction; PE – phycoerytherin; PI – propidium iodide; P-gp – permeability protein.

Minimal residual disease detection. 3-colour flow cytometry was used for MRD detection. Aliquots of bone marrow samples were incubated with monoclonal antibodies (MAb, Becton Dickinson) for 20 min at room temperature in dark. After that, erythrocytes were lysed by FACS Lysing Solution and samples were washed twice in Cell Wash buffer and fixed in paraformaldehyde. The patterns of antigens expression was analyzed with flow cytometer FACSCan (Becton Dickinson) using CellQuestPro software. The following combinations of MAb were used: CD20/CD10/CD19, CD58/CD10/CD19, CD10/CD34/CD19, CD10/CD11a/CD19 and CD45RA/CD10/CD19 conjugated with FITC, PE, PE-Cy5. At least 3 x 10^5 leukocytes were analyzed for each MAb combination. MRD positivity was defined as 0,01% of leukemic cells in a total count of leukocytes in a bone marrow sample [32].

Cell cycle analysis. Leukemic cells of all used samples were isolated from bone marrow by gradient density centrifugation. To study cell cycle distribution leukemic cells were fixed in 70% ethanol. Then cells were washed and treated with RNAse and PI solution [33]. DNA content was evaluated using flow cytometry and ModFit program (Verity Software House).

P-gp expression and function. P-gp expression was evaluated with FITC-labeled 17F9 Mab (BD). P-gp function was tested by accumulation of JC-1 (Molecular Probes) with and without cyclosporine A [34]. P-gp expression and function were analyzed by flow cytometer.

Real-time PCR. Cells were isolated as described in cell cycle analysis paragraph. Total RNA from leukemic cells was extracted using Gen Elute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St Louis, MO, USA). According to manufacturer's protocol after first step (cell lysing) samples were stored at –70°. When needed, samples were thawed and RNA was extracted. Quantity and quality of obtained total RNA were defined with spectrophotometer Gene Quant RNA/DNA Calculator (GE Healthcare). Reaction of reverse transcription was carried out immediately after RNA extraction using Advantage RT-for-PCR Kit (BD) according to the manufacturer's protocol.

We evaluated expression levels of experimental genes using real-time PCR (iCycler, BioRad). For calculating relative expression levels standard curves method was used. Each standard curve was generated from four 10-fold dilutions of cDNA obtained from IM-9 cell line. cDNA synthesized from one extraction of RNA from IM-9 cell line was used for creating standard curves for analyzed genes and as a calibrator (a sample used as the basis for comparative expression results). Each reaction plate contained standard curves for a target gene and a control gene. Normal gene GUS was used as a control gene [35]. cDNA from IM-9 cell line was used for creating the standard curves because it expresses the control and all the target genes. For all experimental samples, target quantity was determined by interpolating from the standard curve and then dividing by the target quantity of the calibrator. The calibrator, then, becomes the 1X sample, and all other quantities are expressed as an n-fold difference relative to the calibrator [36]. To avoid using numbers smaller than 1 (when there is less target RNA in the test sample than in the calibrator), all arbitrary units of gene expression levels in our study represent the fold-difference multiplied by 1000.

Amplifications were carried out in a total volume of 25 µl containing cDNA, Platinum Quantitative PCR SuperMix-UDG (Invitrogene, Carlsbad, CA, USA, final concentration of $MgCl_2$ was raised to 4 mM), 300 nM of forward and reverse primer, and 200 nM of TaqMan probe. The following primers were used (5' to 3'): Bcl-2 forward primer: TTG GCC CCC GTT GCT T, reverse primer: CGG TTG TCG TAC CCC GTT CTC, TaqMan probe: FAM AGC GTG CGC CAT CCT TCC CAG BHQ1; MDR1 forward primer: AGG AAG ACA TGA CCA GGT ATG C, reverse primer: CCA ACA TCG TGC ACA TCA AAC, TaqMan probe: FAM CCT GGC AGC TGG AAG ACA AAT ACA CAA BHQ1; LRP forward primer: CAG CTG GCC ATC GAG ATC A, reverse primer: TCC AGT CTC TGA GCC TCA TGC, TaqMan probe: FAM CAA CTC CCA GGA AGC GGC GGC BHQ1; BCRP forward primer: TGG CTG TCA TGG CTT CAG TA, reverse primer: GCC ACG TGA TTC TTC CAC AA, TaqMan probe: FAM AGC AGG GCA TCG AGC TCT CAC CCT G BHQ1.

Statistical analysis. Results of experiments were presented as the median (lower quartile — upper quartile; valid number of cases tested). We used nonparametric Mann — Whitney U test to compare a certain property between two groups and Spearman Rank order for correlation tests (results presented as R value; P. of significance; valid number of pairs tested). All calculations and graphs were computed using software STATISTICA 6.1.

RESULTS AND DISCUSSION

Results of minimal residual disease monitoring at the end of the induction therapy is thought to be a very informative tool for prediction of a relapse [32]. Thus, we confronted data on drug resistance and apoptosis genes expression levels of all initial samples enrolled into the study with the results of MRD detection on day 36 of the same samples. MRD positivity was defined as 0,01% of leukemic cells in a total count of leukocytes in a bone marrow sample. By this criterion, all studied samples were divided into two groups: MRD positive (MRD^+, n = 9) and MRD negative (MRD^-, n = 10). In MRD^+ group the count of leukemic cells ranged from 0.03% to 0.3% with median at 0.08%. In MDR^- group the quantity of leukemic cells ranged from 0.001% to 0.006% with median at 0.003%. Graphical summary of relative expression levels of studied genes in two groups of patients represented on Figure 1.

Medians of relative expression levels of MDR1 gene (Figure, *a*) were roughly comparable and in MRD^+ group came to 22.8 (0.02–26.6; n = 9) *vs* 24.8 (3.9–41.4; n = 10) in MRD^- group. These results suggest that expression of *MDR1* gene in bulk population of leukemic blasts at diagnosis does not reflect their multidrug resistance potential. Several similar studies confirmed this hypothesis [12, 14, 37]. P-glycoprotein expression may be an adverse

prognostic factor in adult (but not childhood) ALL [10]. We were able to supplement data on *MDR1* gene expression with P-gp expression and function profiles for most studied samples. However, neither function no expression of P-gp protein displayed any significant differences in compared MRD^+ and MRD^- group (data not shown). Probably due to the small group of samples we were also unable to show statistically significant correlation between expression of MDR1 gene and P-gp expression and function.

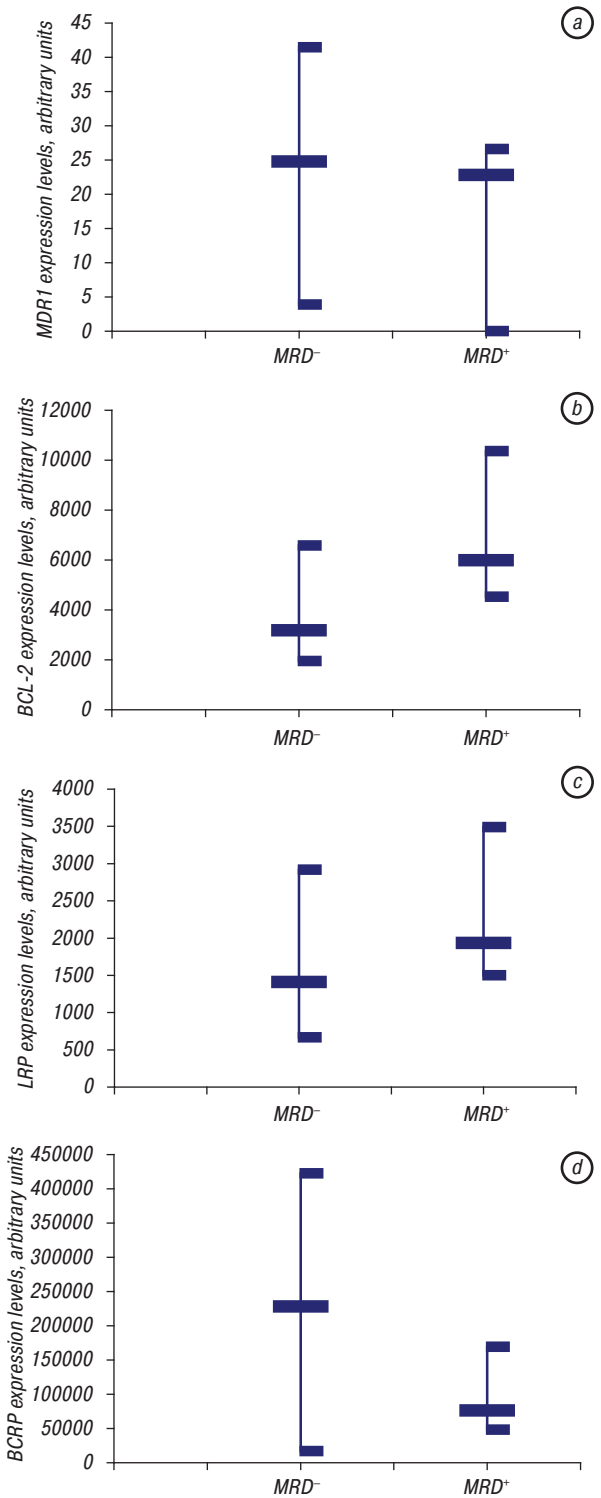

Figure. Relative expression levels of *MDR1* (*a*), *BCL-2* (*b*), *LRP* (*c*) and *BCRP* (*d*) genes in groups of ALL patients with negative (MRD^-) and positive (MRD^+) minimal residual disease at the end of the induction therapy. Medians, 25 and 75 percentiles are shown

According to many researchers, expression of Bcl-2 protein has generally been associated with an adverse prognosis in CLL and AML [38, 39]. Other studies either did not confirm this or, in contrast, showed an improved EFS in childhood ALL [40–42]. Some authors speculated that such controversy may be a result of post-translational modifications of Bcl-2 [30, 43]. According to our study, *Bcl-2* gene (Figure, *b*) showed a tendency ($P = 0.1$) to higher expression levels in MRD^+ group with median at 5992.9 (4521.0–10362.0; n = 9) compared with 3183.6 (1947.9–6581.0; n = 10) in MRD^- group.

Cellular distribution and a putative transport function of vault complexes suggest their role in drug resistance [44]. Therefore, increased expression level of the major vault protein (or lung resistance protein) encoded by *LRP* gene potentially may serve as a marker of a multidrug resistance phenotype. It was shown that reduced intracellular retention of daunorubicin *in vitro* is associated with higher *in vitro* drug resistance and higher *LRP* levels, rather than Pgp or *MRP* expression [11, 17, 18]. Although, other publications showed no association of *LRP* gene expression levels with response to therapy neither in ALL, no in AML patients [12, 27, 28]. In our studies *LRP* gene (Figure, *c*) relative expression levels were similar in both groups and came to 1934.9 (1500.7–3490.4; n = 9) and 1408.5 (665.5–2917.1; n = 10) in MRD^+ and MRD^- group, respectively.

Since its discovery in 1998, the role of expression level of BCRP in AML and ALL have been actively studied with contradictory results [10, 16]. According to some researchers, expression levels of *BCRP* gene in initial and relapsed samples of ALL patients were not different, and there were no association between this gene overexpression and unfavorable prognosis [15]. According to our data, the median of *BCRP* gene (Figure, *d*) expression levels in MRD^+ group was considerably lower than that in MRD^- group, namely 76000.0 (48196.2–169230.8; n = 9) and 227967.2 (16683.7–422222.2; n = 10), respectively, but statistical analysis showed no significant difference for this parameter.

According to recent publication, higher proliferating activity within initial samples of ALL correlated with an increased drug sensitivity [45]. Similarly, higher complete remission rate in AML was associated with high proliferative activity [46]. Nevertheless, these findings contradict with some earlier publications [47, 48]. Cell cycle distribution tests performed for present investigation showed a tendency ($P = 0.15$) to lower percentage of cells in S + G2M phase in MRD^+ group: 10.1 (3.9–12.1; n = 7) compared with 13.5 (8.2–17.2; n = 6) in MRD^- group.

In conclusion, we investigated expression of multidrug resistance and apoptosis related genes in leukemic cells at diagnosis and MRD level at the end of induction therapy and could not find obvious relations between these parameters. Larger study is required to prove these results, although similar investigations showed association between an apoptosis-resistant protein profile, Pgp activity and MRD level in AML [49, 50]. It is also possible, that treatment response

depends on the expression of multidrug resistance and apoptosis associated genes not in total population but in the putative leukemic stem cells population instead [51]. Apparently, identification of intrinsic qualities of chemoresistant cells would lead to a better understanding of mechanisms of drug resistance and may potentially give more opportunities for targeted therapy development.

REFERENCES

1. **Uderzo C, Conter V, Dini G, *et al.*** Treatment of childhood acute lymphoblastic leukemia after the first relapse: curative strategies. Haematologica 2001; **86**: 1–7.

2. **Borst P.** Genetic mechanisms of drug resistance. A review. Acta Oncol 1991; **30**: 87–105.

3. **Kruh GD.** Introduction to resistance to anticancer agents. Oncogene 2003; **22**: 7262–4.

4. **Beck J, Handgretinger R, Dopfer R, *et al.*** Expression of mdr1, mrp, topoisomerase II alpha/beta, and cyclin A in primary or relapsed states of acute lymphoblastic leukaemias. Br J Haematol 1995; **89**: 356–63.

5. **Dhooge C, De Moerloose B, Laureys G, *et al.*** P-glycoprotein is an independent prognostic factor predicting relapse in childhood acute lymphoblastic leukaemia: results of a 6-year prospective study. Br J Haematol 1999; **105**: 676–83.

6. **Dhooge C, De Moerloose B, Laureys G, *et al.*** Expression of the multidrug transporter P-glycoprotein is highly correlated with clinical outcome in childhood acute lymphoblastic leukemia: results of a long-term prospective study. Leuk Lymphoma 2002; **43**: 309–14.

7. **De Moerloose B, Swerts K, Benoit Y, *et al.*** The combined analysis of P-glycoprotein expression and activity predicts outcome in childhood acute lymphoblastic leukemia. Pediatr Hematol Oncol 2003; **20**: 381–91.

8. **Casale F, D'Angelo V, Addeo R, *et al.*** P-glycoprotein 170 expression and function as an adverse independent prognostic factor in childhood acute lymphoblastic leukemia. Oncol Rep. 2004; **12**: 1201–7.

9. **Polgar O, Bates SE.** ABC transporters in the balance: is there a role in multidrug resistance? Biochem Soc Trans 2005; **33**: 241–5.

10. **Steinbach D, Legrand O.** ABC transporters and drug resistance in leukemia: was P-gp nothing but the first head of the Hydra? Leukemia 2007; **21**: 1172–6.

11. **den Boer M, Pieters R, Kazemier K, *et al.*** Relationship between major vault protein/lung resistance protein, multidrug resistance-associated protein, P-glycoprotein expression, and drug resistance in childhood leukemia. Blood 1998; **91**: 2092–8.

12. **Kakihara T, Tanaka A, Watanabe A, *et al.*** Expression of multidrug resistance-related genes does not contribute to risk factors in newly diagnosed childhood acute lymphoblastic leukemia. Pediatr Int 1999; **41**: 641–7.

13. **Stam R, van den Heuvel-Eibrink M, den Boer M, *et al.*** Multidrug resistance genes in infant acute lymphoblastic leukemia: Ara-C is not a substrate for the breast cancer resistance protein. Leukemia 2004; **18**: 78–83.

14. **Valera E, Scrideli C, Queiroz R, *et al.*** Multiple drug resistance protein (MDR-1), multidrug resistance-related protein (MRP) and lung resistance protein (LRP) gene expression in childhood acute lymphoblastic leukemia. Sao Paulo Med J 2004; **122**: 166–71.

15. **Sauerbrey A, Sell W, Steinbach D, *et al.*** Expression of the BCRP gene (ABCG2/MXR/ABCP) in childhood acute lymphoblastic leukaemia. Br J Haematol 2002; **118**: 147–50.

16. **Robey R, Polgar O, Deeken J, *et al.*** ABCG2: determining its relevance in clinical drug resistance. Cancer Metastasis Rev 2007; **26**: 39–57.

17. **Den Boer M, Pieters R, Kazemier K, *et al.*** Relationship between the intracellular daunorubicin concentration, expression of major vault protein/lung resistance protein and resistance to anthracyclines in childhood acute lymphoblastic leukemia. Leukemia 1999; **13**: 2023–30.

18. **Volm M, Stammler G, Zintl F, *et al.*** Expression of lung resistance-related protein (LRP) in initial and relapsed childhood acute lymphoblastic leukemia. Anticancer Drugs 1997; **8**: 662–5.

19. **Kedersha N, Rome L.** Vaults: large cytoplasmic RNP's that associate with cytoskeletal elements. Mol Biol Rep 1990; **14**: 121–2.

20. **Hamill D, Suprenant K.** Characterization of the sea urchin major vault protein: a possible role for vault ribonucleoprotein particles in nucleocytoplasmic transport. Dev Biol 1997; **190**: 117–28.

21. **Herrmann C, Golkaramnay E, Inman E, *et al.*** Recombinant major vault protein is targeted to neuritic tips of PC12 cells. J Cell Biol 1999; **144**: 1163–72.

22. **Abbondanza C, Rossi V, Roscigno A, *et al.*** Interaction of vault particles with estrogen receptor in the MCF-7 breast cancer cell. J Cell Biol 1998; **141**: 1301–10.

23. **Chugani D, Rome L, Kedersha N.** Evidence that vault ribonucleoprotein particles localize to the nuclear pore complex. J Cell Sci 1993; **106**: 23–9.

24. **Goasguen J, Lamy T, Bergeron C, *et al.*** Multifactorial drug-resistance phenomenon in acute leukemias: impact of P170-MDR1, LRP56 protein, glutathione-transferases and metallothionein systems on clinical outcome. Leuk Lymphoma 1996; **23**: 567–76.

25. **Hart S, Ganeshaguru K, Scheper R, *et al.*** Expression of the human major vault protein LRP in acute myeloid leukemia. Exp Hematol 1997; **25**: 1227–32.

26. **Xu D, Areström I, Virtala R, *et al.*** High levels of lung resistance related protein mRNA in leukaemic cells from patients with acute myelogenous leukaemia are associated with inferior response to chemotherapy and prior treatment with mitoxantrone. Br J Haematol 1999; **106**: 627–33.

27. **Legrand O, Simonin G, Zittoun R, *et al.*** Lung resistance protein (LRP) gene expression in adult acute myeloid leukemia: a critical evaluation by three techniques. Leukemia 1998; **12**: 1367–74.

28. **Leith C, Kopecky K, Chen I, *et al.*** Frequency and clinical significance of the expression of the multidrug resistance proteins MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia: a Southwest Oncology Group Study. Blood 1999; **94**: 1086–99.

29. **Michieli M, Damiani D, Ermacora A, *et al.*** P-glycoprotein, lung resistance-related protein and multidrug resistance associated protein in de novo acute non-lymphocytic leukaemias: biological and clinical implications. Br J Haematol 1999; **104**: 328–35.

30. **Del Principe M, Del Poeta G, Maurillo L, *et al.*** P-glycoprotein and BCL-2 levels predict outcome in adult acute lymphoblastic leukaemia. Br J Haematol 2003; **121**: 730–8.

31. **Uckun F, Yang Z, Sather H, *et al.*** Cellular expression of antiapoptotic BCL-2 oncoprotein in newly diagnosed childhood acute lymphoblastic leukemia: a Children's Cancer Group Study. Blood 1997; **89**: 3769–77.

32. **Dworzak M, Fröschl G, Printz D, *et al.*** Austrian Berlin-Frankfurt-Münster Study Group. Prognostic significance and modalities of flow cytometric minimal residual disease

detection in childhood acute lymphoblastic leukemia. Blood 2002; **99**: 1952–8.

33. **Ormerod M.** Analysis of DNA: general methods. In: Ormerod MG, ed. Flow cytometry: a practical approach. New York: Oxford University Press; 1994: 119–135.

34. **Legrand O, Perrot J, Simonin G, *et al.*** JC-1: a very sensitive fluorescent probe to test Pgp activity in adult acute myeloid leukemia. Blood 2001; **97**: 502–8.

35. **Beillard E, Pallisgaard N, van der Velden V, *et al*.** Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) — a Europe against cancer program. Leukemia 2003; **17**: 2474–86.

36. Applied Biosystems Instruction Manuals, Section VII, Relative Quantitation of Gene Expression; Experimental Design and Analysis: Relative Standard Curve Method and Comparative Ct. Method, P 34–43.

37. **Kanerva J, Tiirikainen M, Mäkipernaa A, *et al.*** Initial P-glycoprotein expression in childhood acute lymphoblastic leukemia: no evidence of prognostic impact in follow-up. Pediatr Hematol Oncol 2001; **18**: 27–36.

38. **Robertson L, Plunkett W, McConnell K, *et al.*** Bcl-2 expression in chronic lymphocytic leukemia and its correlation with the induction of apoptosis and clinical outcome. Leukemia 1996; **10**: 456–9.

39. **Campos L, Rouault J, Sabido O, *et al.*** High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. Blood 1993; **81**: 3091–6.

40. **Schimmer A, Hedley D, Penn L, *et al.*** Receptor- and mitochondrial-mediated apoptosis in acute leukemia: a translational view. Blood 2001; **98**: 3541–53.

41. **Coustan-Smith E, Kitanaka A, Pui C, *et al.*** Clinical relevance of BCL-2 overexpression in childhood acute lymphoblastic leukemia. Blood 1996; **87**: 1140–6.

42. **Wuchter C, Karawajew L, Ruppert V, *et al.*** Constitutive expression levels of CD95 and Bcl-2 as well as CD95 function and spontaneous apoptosis in vitro do not predict the response to induction chemotherapy and relapse rate in childhood acute lymphoblastic leukaemia. Br J Haematol 2000; **110**: 154–60.

43. **Reed J.** Bcl-2-family proteins and hematologic malignancies: history and future prospects. Blood 2008; **111**: 3322–30.

44. **Mossink M, van Zon A, Scheper R, *et al.*** Vaults: a ribonucleoprotein particle involved in drug resistance? Oncogene 2003; **22**: 7458–67.

45. **Kaaijk P, Kaspers G, Van Wering E, *et al.*** Cell proliferation is related to in vitro drug resistance in childhood acute leukaemia. Br J Cancer 2003; **88**: 775–81.

46. **Braess J, Jahns-Streubel G, Schoch C, *et al.*** Proliferative activity of leukaemic blasts and cytosine arabinoside pharmacodynamics are associated with cytogenetically defined prognostic subgroups in acute myeloid leukaemia. Br J Haematol 2001; **113**: 975–82.

47. **Smets L, Slater R, van Wering E, *et al.*** DNA index and %S-phase cells determined in acute lymphoblastic leukemia of children: a report from studies ALL V, ALL VI, and ALL VII (1979–1991) of the Dutch Childhood Leukemia Study Group and The Netherlands Workgroup on Cancer Genetics and Cytogenetics. Med Pediatr Oncol 1995; **25**: 437–44.

48. **Vidriales M, Orfao A, López-Berges M, *et al.*** Prognostic value of S-phase cells in AML patients.Br J Haematol 1995; **89**: 342–8.

49. **van Stijn A, Feller N, Kok A, *et al.*** Minimal residual disease in acute myeloid leukemia is predicted by an apoptosis-resistant protein profile at diagnosis. Clin Cancer Res 2005; **11**: 2540–6.

50. **van der Pol M, Feller N, Ossenkoppele G, *et al.*** Minimal residual disease in acute myeloid leukemia is predicted by P-glycoprotein activity but not by multidrug resistance protein activity at diagnosis. Leukemia 2003; **17**: 1674–7.

51. **Ho M, Hogge D, Ling V.** MDR1 and BCRP1 expression in leukemic progenitors correlates with chemotherapy response in acute myeloid leukemia. Exp Hematol 2008; **36**: 433–42.

ЭКСПРЕССИЯ ГЕНОВ *MDR1, LRP, BCRP* И *BCL-2* ПРИ УСТАНОВЛЕНИИ ДИАГНОЗА ОЛЛ У ДЕТЕЙ: СРАВНЕНИЕ С РЕЗУЛЬТАТАМИ МОНИТОРИНГА МИНИМАЛЬНОЙ РЕЗИДУАЛЬНОЙ БОЛЕЗНИ ПОСЛЕ ИНДУКЦИОННОЙ ТЕРАПИИ

Цель: **изучить свойства лейкозных клеток детей больных ОЛЛ с положительным и отрицательным результатом мониторинга минимальной резидуальной болезни после индукционной терапии.** ***Методы*****: согласно результатам мониторинга минимальной резидуальной болезни (MRD) с помощью трехцветной проточной цитометрии на 36-й день больные были разделены на 2 группы: MRD^- и MRD^+. Для изучения уровней экспрессии генов *MDR1, LRP, BCRP* и *BCL-2* в образцах костного мозга пациентов с диагнозом первичного ОЛЛ использовался метод относительной количественной ПЦР в режиме реального времени.** ***Результаты:*** **медианы уровней экспрессии гена *MDR1* в изученных группах пациентов отличались незначительно и составили 22,8 (0,02–26,6; n = 9) в MRD^+ и 24,8 (3,9–41,4; n = 10) в MRD^--группе. Тенденция к повышенной экспрессии гена *BCL-2* выявлена в группе MRD^+, где медиана составила 5992,9 (521,0–10362,0; n = 9), по сравнению с 3183,6 (1947,9–6581,0; n = 10) в группе MRD^-. Относительная экспрессия гена *LRP* в изучаемых группах оказалась сходной, медианы составили 1934,9 (1500,7–3490,4; n = 9) и 1408,5 (665,5–2917,1; n = 10) в MRD^{+-} и MRD^--группах соответственно. Медиана уровня экспрессии гена *BCRP* в группе MRD^+ была значительно ниже таковой в группе MRD^- — 76000,0 (48196,2–169230,8; n = 9) и 227967,2 (16683,7–422222,2; n = 10) соответственно, однако статистических анализ не подтвердил значимость различий.** ***Выводы:*** **при изучении уровней экспрессии генов *MDR1, LRP, BCRP* и *BCL-2* в лейкозных клетках при установлении первичного диагноза ОЛЛ у детей и при мониторинге MRD после индукционной терапии не выявлена связь между данными параметрами.**

Ключевые слова**: ОЛЛ, МРБ, BCL-2, MDR1, LRP, BCRP.**