Use of microarrays and MLPA for integrating diagnostics and personalizing treatment – Case report of a patient with Ph-like acute B-cell lymphoblastic leukemia

Monika Lejman[1,A,C-D,F] , Monika Włodarczyk[2,C-D,F] , Agnieszka Zaucha-Prażmo[3,B,F] ,
Joanna Zawitkowska[3,B,F]

[1] Laboratory of Genetic Diagnostics, Department of Paediatric Haematology, Oncology and Transplantology, Medical University, Lublin, Poland
[2] Laboratory of Genetic Diagnostics, Medical Univesity, Lublin, Poland
[3] Department of Paediatric Haematology, Oncology and Transplantology, Medical University, Lublin, Poland
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation,
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Abstract
B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common childhood cancer. A special subtype of high risk BCP-ALL is Philadelphia-like ALL (Ph-like ALL), in which the gene expression profile is similar to *BCR-ABL1*-positive leukemia; however, fusion of the mentioned genes does not occur. The unfavourable clinical course and incidence of 15% of cases means that the diagnosis and therapeutic strategy of Ph-like ALL must be carefully developed and implemented into clinical practice. The study presents the case of a patient with diagnosed Ph-like ALL. The use of molecular analytical techniques has made it possible to identify a patient who is likely to relapse and who may benefit from personalized therapy This study shows the advantages of using genomic analyses to identify therapeutic targets, which is especially important for patients with high-risk disease. This model of management could be extended to other cancer subtypes, allowing for tailored diagnosis.

Key words
acute lymphoblastic leukemia, Philadelphia-like ALL, genetic aberrations, molecular abnormalities

INTRODUCTION

B-cell precursor acute lymphoblastic leukemia is the most common type of leukemia in childhood – it accounts for about 80% of leukemia in this age group. Complete remission is achieved in >80% of patients; however, about 20% of patients relapse. Chemical resistance and an increase in the proliferative potential of abnormal lymphoblasts in relapse contribute to a worse response to treatment. Relapse of ALL in children is often associated with changes in lymphoblast morphology and immunophenotype; more complex cytogenetic rearrangements are also observed [1, 2, 3, 4].

Acute lymphoblastic leukemia is a heterogeneous group of cancer diseases, and its numerous genetic changes are the basis for the division of ALL into specific subtypes. Individual ALL subtypes differ from each other by many features, e.g., chromosomal and gene aberrations, immunophenotype and cytomorphology. Cytogenetic studies and gene expression analysis using the microarray technique allow patients to be classified into appropriate risk groups [1].

Address for correspondence: Monika Lejman, Laboratory of Genetic Diagnostics, Department of Paediatric Haematology, Oncology and Transplantology, Medical University, Lublin, Poland
E-mail: monika.lejman@umlub.pl

CASE REPORT

A 9-year-old boy was admitted to the Department of Paediatric Haematology, Oncology and Transplantology at the Medical University in Lublin, eastern Poland, due to a fever lasting for 7 days, bone pain and weakness. Lymphadenopathy, hepatosplenomegaly and the infiltration of the central nervous system were not observed. Complete peripheral blood count revealed: WBC 6800/µl; HB = 9.1g/dl; PLT = 77000/µl. Undifferentiated cells were not detected in the peripheral blood, while 91.2% of the blasts were found in the bone marrow. The child was diagnosed with B-cell precursor common positive ALL and chemotherapy was started in April 2015, according to ALL IC-BFM 2009 (ALL Intercontinental-BFM 2009) protocol. He was classified to the intermediate risk group (IRG). 24-hour unstimulated culture was performed to assess the somatic karyotype. GTG band staining and FISH (fluorescent *in situ* hybridizatio*n*) tests were performed using molecular probes: *BCR/ABL1*, *KMT2A*, *ETV6/ RUNX1* (Vysis, Abbott Molecular, Illinois, USA). No rearrangement was found, and the signal arrangement from the other probes was correct. The patient had a normal somatic karyotype 46, XY. He completed the first line therapy in November 2016. The relapse has occurred 11 months later. The bone marrow and testicular infiltration were observed. Therapy was started according to the IntReALL-SR-2010 (International Study for Treatment of Standard
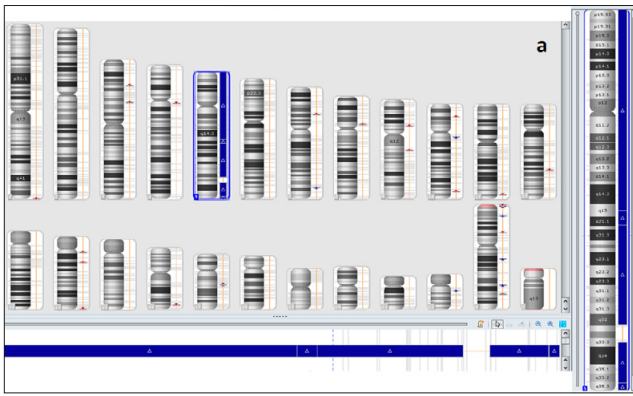
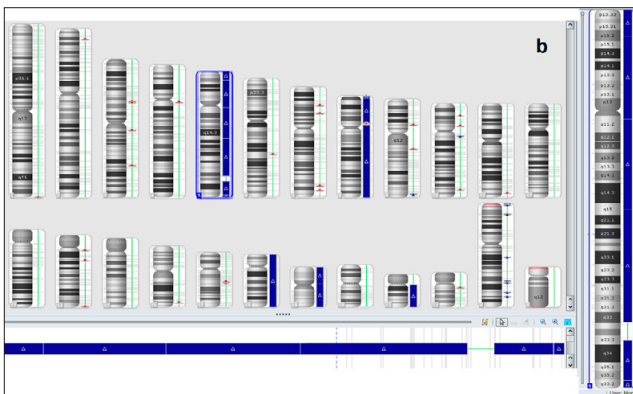**Figure 1A.** Microarray results at the time of diagnosis

Figure 1B. Microarray results at relapse

Figure 2A and 2B. Microarray results showing rupture within *CSF1R* last exon, whole *PDGFRB* gene and *EBF1* first exon in additional chromosome 5 derivative

Risk Childhood Relapsed ALL 2010). He received 4 blocks of chemotherapy before haematopoietic cell transplantation and achieved disease remission. Genetic tests were performed again using classical cytogenetics and FISH. There was found hyperdiploid karyotype: 51,XY,+5,+8,+18,+19,+21. No fusion genes (*BCR/ABL1, KMT2A, ETV6/RUNX1*) were revealed. Additional tests were then performed using the MLPA technique (P036-E3, P335-C1, MRC Holland, Amsterdam, The Netherlands).

The results of the study indicated abnormalities within the chromosome 5. Additional test was performed using the CytoScan HD microarray technique (2 670 000 probes including 750 000 SNPs; Thermo Fischer, Waltham, M, USA) in leukemic cell from diagnosis and relapse (Fig. 1A and 1B). An additional marker chromosome derived from chromosome 5 was found in samples from diagnosis and relapse (Fig. 1A and 1B). In an additional chromosome 5 derivative, rupture within *CSF1R* last exon, whole *PDGFRB* gene and *EBF1* first exon were identified (Fig. 2). Microarray test confirmed Trisomies chromosomes 8, 18, 19 and 21 in a sample from relapse. Break apart probes (CytoCell, Cytocell Ltd, Oxford Gene Technology, Cambridge, UK) were used to confirm rearrangement within the *CSF1R* gene (Fig. 3A, 3B and 3C). A positive result was obtained. The patient was qualified for haematopoietic cell transplantation. At present, the patient is alive, without disease recurrence, two years after transplantation.

DISCUSSION

Due to the multitude of genetic rearrangements associated with BCP-ALL, the determination of genomic abnormalities is an important prognostic indicator and enables the stratification of patients. Chromosome aberrations in the form of deletions or duplications relate to genes involved in the process of leukemogenesis at various levels. The number of point mutations or deletions in BCP-ALL may increase as the disease progresses. This is particularly important in the event of relapse, as it may lead to an intensification of the adverse prognostic effect, thus limiting the response to the therapy used [5, 6].

The described clinical case is characterized by a gene expression profile analogous to ALL with the *BCR-ABL1* fusion in the absence of this chromosomal aberration. This ALL subtype, recognized as Philadelphia-like ALL, accounts for 15% of BCP-ALL cases. The described phenotype of the disease is also recognized in ALL in adults – it accounts for >20% of cases, with an emphasis on young adults, where the morbidity reaches up to 27%. The 5-year survival rate is 54%, including 25.8% for young adults, 65.8% for adolescents and 72.8% for HR children [7].

Characteristic for Ph-like ALL is the high risk of relapse, poor response to chemotherapy and poor outcome. Diagnosis of *BCR-ABL*-like ALL is possible using molecular microarrays that identify abnormalities within the genome of lymphoid cells. Also, in the case of Ph-like ALL, most chromosomal aberrations refer to genes involved in leukemogenesis (*IKZF1, PAX5, EBF1, CRLF2, CDKN2A/2B*) [8, 9, 10, 11].

However, the described clinical case concerns a relatively rare type of abnormality characteristic of BCR-ABL-like ALL. Approximately 14% of Ph-like mutations are changes (mainly deletions) within the *EBF1* gene, which is
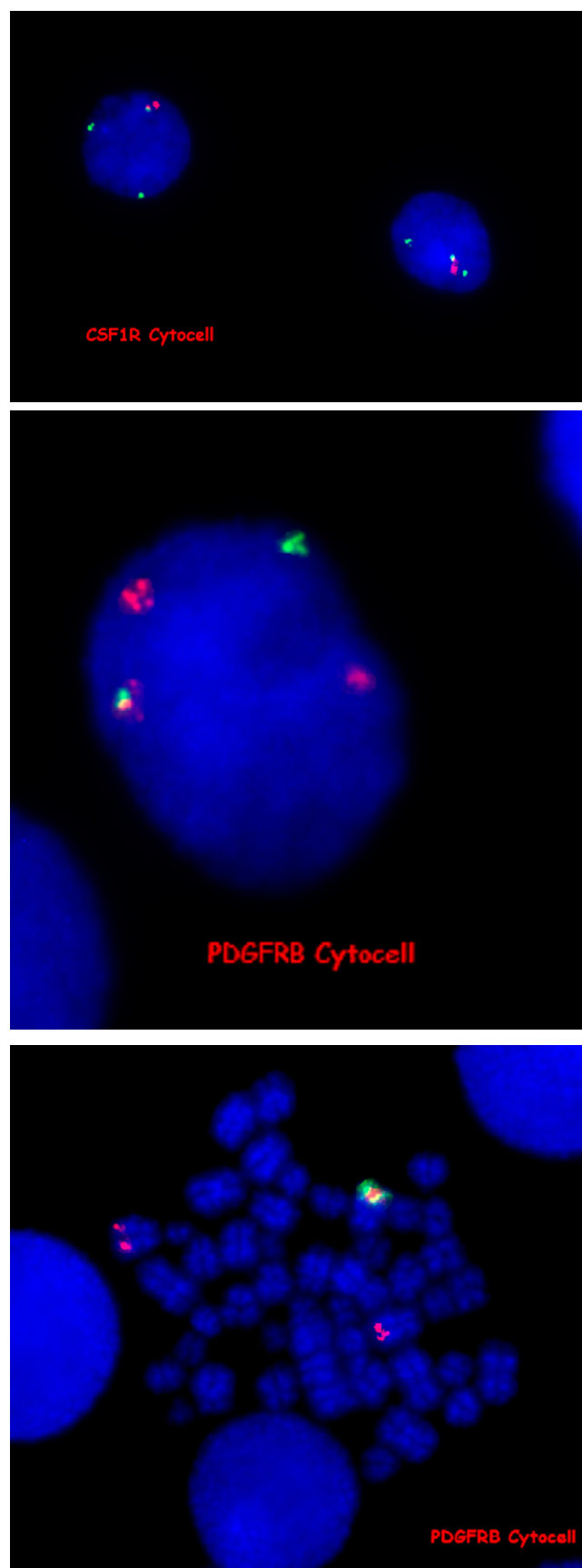
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Figure 3A, 3B, 3C. Images of interphase FISH with *CSF1R* and *PDGFRB* probes. FISH was performed on cells in interphase using the *CSF1R* and *PDGFRB* Break Apart probe (Cytocell Ltd, Oxford Gene Technology, Cambridge, UK), according to the manufacturer's recommendations. Images were captured by an Olympus microscope and analyzed with Isis Software (MetaSystems)

a transcription factor involved in the B-cell differentiation process. In turn, the genetic alterations responsible for excessive kinase signalling are characteristic for about 90% of Ph-like ALL cases. They relate to kinases, cytokines or cytokine receptor genes, which include, but are not limited to: *ABL1*, *CRLF2*, *CSF1R*, *PDGFRB*, and *EPOR*. *EBF1* is a fusion partner for *PDGFRB*, while there are no reports of a relationship between the *EBF1* fragment deletion and *CSF1R* exon 1 deletion. The colony-stimulating factor 1 receptor (*CSF1R*) codes for tyrosine kinase transmembrane receptor responsible for macrophage proliferation and differentiation. The only fusion partner described to date for *CSF1R* is the *SSBP2* gene. The incidence of this fusion for Ph-like ALL is around 2.6%. *CSF1R-SSBP2* fusion may occur, *inter alia*, by chromothripsis of chromosome 5. Studies using cell cultures showed that cells with *CSFR1-SSBP2* fusion were sensitive to the tyrosine kinase inhibitors – imatinib and dasatinib. In addition, *CSF1R* inhibitors are a promising class of immunomodulatory drugs that, when used in combination with cancer immunotherapy agents, help to achieve clinical benefits for patients [9, 12, 13, 14].

In Poland, there are no procedures developed to diagnose Ph-like ALL. Criteria in the form of a set of genes whose over-expression is characteristic of this BCP-ALL subtype have not been developed to-date. Changes involving kinase signalling affect up to 90% of patients in Ph-like ALL. In the case of more changes resulting in the activation of kinases, the use of TKI is an effective form of therapy. Personalization of treatment preceded by rapid diagnostics will be possible after a comprehensive analysis of the genetic basis, including the identification of signalling pathways and cytokine receptors involved in the process of tumour transformation. Only the use of molecular analytical tools – next generation sequencing or microarrays – will allow improvement in the diagnosis of Ph-like ALL [15, 16, 17].

CONCLUSIONS

The use of a high-throughput analytical technique in the described case allowed identification of a patient with a particularly aggressive form of leukemia; thus, he may benefit from targeted therapy (imatinib, dasatinib). Understanding the molecular mechanisms that lead to neoplastic transformation of lymphoblasts will allow the development of an appropriate diagnostic and therapeutic system. The study of genetic profiles using sensitive analytical techniques will allow the creation of 'molecular portraits' of cancer cells and personalization of treatment. This model of management could be extended to other cancer subtypes, allowing for tailored diagnosis.

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