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CASE REPORT

A NEW CASE OF CHILDHOOD ACUTE LYMPHOBLASTIC B-CELL LEUKEMIA FROM PRISTINA

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Abstract. *Acute lymphoblastic leukemia (ALL) is a malignant disease caused by mutations in B- or T-cell precursors of bone marrow cells. Childhood acute lymphoblastic leukemia (ALL) is a subtype of pediatric cancer with a 1 in 2000 incidence. Here we present a new childhood ALL in a 3-year-old girl. As CD45/19, CD10/19, CD3, CD8, CD10, and CD19 were positive in immunohistochemically analyses of blast cells, a B-ALL was diagnosed with a causative ETV6-RUNX1 gene fusion. The patient was treated based on standard protocols BMF-ALL 2009. Interestingly, an aunt and a grandfather of the patient had experienced malignancies as well, which may be carefully interpreted as a hint on a familial cancer syndrome.*

Key words: *acute lymphoblastic leukemia, cancer syndrome, childhood gene fusion*

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) accounts for 30% of cancer diagnoses in children under the age of 15 years [1], with a cumulative risk of 1 in 2,000 children up to the age of 15 years [1]. The disease is diverse, considering both biological and clinical aspects, presenting different subtypes; each subtype has been shown to be associated with various genetic alternations with own prognostic relevance [2]. The most common chromosomal rearrangement observed in ALL is the translocation t(12;21)(p13.2;q22.1) leading to an ETV6-RUNX1 gene fusion [3, 4]. During recent decades, a significant improvement in treatment was achieved being due to such factors as intensive chemotherapy, targeted therapy with monoclonal antibodies, and hematopoietic stem cell transplantation. However, in adults with B-ALL, prognosis remains inferior, as those have adverse outcomes and survival rates decrease with age, being as low as 10-20% in the elderly [5].

To the best of our knowledge, this case with gene fusion *ETV6-RUNX1* due to a translocation t(12;21) presented here is the first childhood B-ALL case diagnosed and treated in Kosovo.

CASE PRESENTATION

A 3-year-old female presented with weakness, headache, nausea, vomiting, and fever. She was the fourth child from the fourth pregnancy of her parents; pregnancy completed at term, with birth weight of 3,900 g.

There was normal development until actual hospitalization. Body weight at time of diagnosis was 20.5 kg, and she showed normal psychomotor development. However, the skin was very pale without turgor efflorescence and preserved elasticity. Liver and spleen were not palpable. The family history indicated that the patient's paternal grandfather had had malignant lung disease, and the patient's paternal aunt had breast cancer (Figure 1).

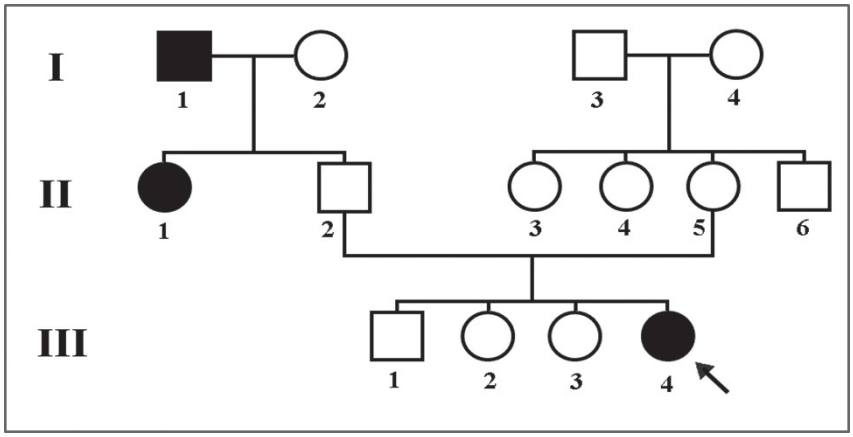

Fig. 1. Pedigree of the patient with childhood ALL (arrow, III-4). Grandfather (I-1) was affected with lung cancer, an aunt (II-1) had breast cancer. Remainder family members were not affected by cancer, yet

Flow cytometry/FACS analysis of peripheral blood samples was positive for cluster differentiation CD45/19, CD10/19, CD3, CD8, CD10, and CD19, but negative for CD20, CD22, CD2, CD7, CD56, CD13, CD14, CD15, CD30, CD33, CD34, and sIgM (Table 1). A reverse transcription (RT-) PCR based molecular screening for gene fusions typically observed in ALL (*BCR-ABL; MLL-AF4; ETV6-RUNX1, E2A-PBX1*) and AML (*MLL-AF4; MLL-AF9; MLL-ENL; RUNX1-RUNX1T1; PML-RARA; CBFb-MYH11*) according to the methods developed by Europe Against Cancer Program, showed that the present case had a *ETV6-RUNX1* gene fusion. Banding cytogenetics revealed a karyotype 46,XX, which means the translocation t(12;21)(p13.2;q22.1) was cryptic, as being typical for this rearrangement. Immunohistology was positive for Pax-5, and immunological staining turned out positive for CD99, CD10, and TdT, with 80% of infiltrates with ALL-blast of L2 morphology. Laboratory analysis of erythrocytes identified anisochromia and hyperchromia; leukocytes expressed leukopenia, neutrophils were absent; the number of thrombocytes was enhanced; granulocytes were much reduced apart from some eosinophils. Biochemical data obtained during the ten days of hospitalization are shown in. Immunological analysis showed that ANA (anti-nuclear antibody), MPO (pANA – perinuclear anti-neutrophil cytoplasmic antibody), and Pr3 (cANCA – antineutrophil cytoplasmic antibody) were negative (Table 1). Bone marrow aspiration has shown morphological changes in blast which are presented in Figure 2.

Table 1. Results of immunological analysis for our patient

Analysis	Results	Reference values	Unit
ANA (Anti-Nuclear Antibody)	0.2 Negative	< 0.8 COI Negative	COI
		0.8-1.2 COI suspected	
		> 1.2 COI Positive	
MPO (pANCA)	<3.0 Negative	< 12 AU/ml Negative	AU/ml
		12-18 AU/ml suspected	
		> 18 AU/ml Positive	
Pr3 (cANCA)	<3.0 Negative	< 12 AU/ml Negative	AU/ml
		12-18 AU/ml suspected	
		> 18 AU/ml Positive	
Complement C3	186.8H	90-180	mg/Dl
Complement C4	29.7	Oct-40	mg/Dl
Immunoglobulin E (IgE)	55.5	< 100	U/Ml
Immunoglobulin A (IgA)	1.8	0.7-4.0	g/L
Immunoglobulin M (IgM)	0.9	0.4-2.3	g/L
Immunoglobulin G (IgG)	10.3		g/L

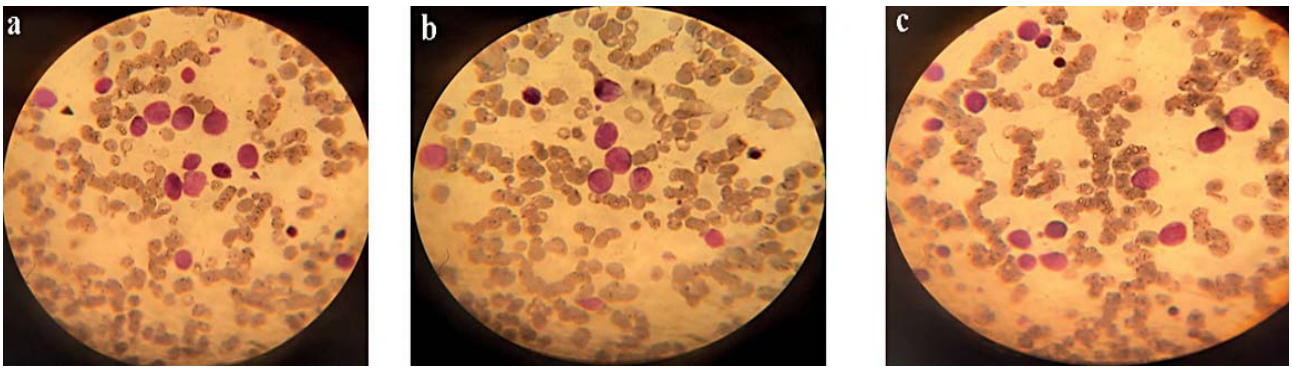

Fig. 2. Bone marrow aspiration was done twice; blasts were below 25% at initial punction, however, 1 month later leukemia was diagnosed. L2 cell morphology (according to FAB classification) are large cells, with heterogeneous chromatin, less cytoplasm, larger nuclei and irregular shape

Our patient was treated according to the AIEOP-BFM ALL 2009 protocol, with some modification (first phase: 6-mercaptopurine 20 mg/m^2/dl; methotrexate 12 mg; leucoverin-rescue i.v. 15 mg/m^2; second phase: prednisone 48 mg; vincristine 1.2 mg; daunorubicin 24 mg; methotrexate 12 mg; third phase: cyclophosphamide 80 mg; ARA-C, cytarabine 75 mg/m^2/d; 6-mercaptopurine 48 mg/m^2/dl; methotrexate (MTX) 12 mg. According to the protocol, (MTX) should be given 5 g/m^2 and we have given 3 g/m^2. Since MTX should be measured in serum and based on serum, MTX values the antidote Amp Leucoverin should be given. Due to the high toxicity of the drug we started with the dose 1/m^2, then 2 g/m^2 and finally we now have 3 g/m^2. We will gradually increase the dose to 5 g/m^2 for other patients; Daunorubicin has been replaced with Doxorubicin because we do not have it on the market in Kosovo; Peg-Asparaginase has been replaced by L-Asparaginase due to its high toxicity. All these changes were made in consultation with two European University Clinics: University Clinic of Graz – LKH and the University Clinic of the Netherlands in Utrecht. She underwent the induction phase with chemotherapy. The patient is now in the consolidation phase with chemotherapy. In the induction phase on day 33, the bone marrow was punctured and resulted in 0% blasts, an indication for a complete remission being achieved.

DISCUSSION

Acute lymphoblastic leukemia (ALL) is a common pediatric malignancy. It is caused by bone marrow abnormalities leading to massive production of white blood cells being harmful for other bone marrow cells and leading to anemia, thrombocytopenia, and even neutropenia. The classic symptoms of children with ALL are fever, easy bruising or bleeding, flat, pinpoint, dark-red spots under the skin caused by bleeding, weakness, feeling tired, or looking pale, bone or joint pain, shortness of breath, painless lumps in the neck, underarm, stomach, or groin, pain or feeling of fullness below the ribs, and loss of appetite [6]. From data obtained in Japan, the male sex is slightly predominant (1.2-fold), with a peak incidence at 1-4 years of age [7]. During the last years, clinical course of childhood ALL improved with an overall surveillance rate exceeding 90%.

Different genetic factors identified by conventional karyotyping can be used for risk stratification. Hyper- and hypodiploidy and several other specific chromosomal rearrangements are typically observed in childhood ALL. Genomic studies furthermore demonstrated a close connection of inherited and somatic genetic alternations in ALL [8]. Diagnoses occur once symptoms appear and include physical examination, complete blood cell count, and bone marrow biopsy. The latter are assessed by microscope for morphological changes and in parallel undergo cultivation, cytogenetic preparation, and analyses for chromosomal changes, which can be supplemented by fluorescence in situ hybridization (FISH) and/or molecular analyses like RT-PCR [9, 10].

Family risk for ALL were assessed when any member of family were diagnosed with any kind of cancer [11]. During the genetic consult, parents reported that the paternal grandfather had suffered from pulmonary cancer and an aunt from breast cancer. Interestingly, in rare cases a familial predisposition for leukemia and other cancer types has been reported: different variants including TP53 mutation in germline (LiFraumeni-syndrome), ETV6 variants and hyperdiploidy in ALL, or PAX5 mutation and B-ALL with dicentric/ischoromosome 9 [12, 13, 14, 15]. Thus, here a familial component predisposing for malignancy cannot be excluded.

In our case, a child with karyotype 46,XX was diagnosed with B-ALL. Together with RT-PCR-result, she had a cryptic translocation t(12;21)(p13.2;q22.1). Previous studies showed that patients with this kind of translocation do extremely well. BFM group showed that the incidence of translocation t(12;21) in relapse cases was identical to that seen at the initial phase of diagnosis [16].

In conclusion, our study underscored the Pession hypothesis that treatment of patients with B-ALL based on BFM-ALL 2009 protocols, with four drug inductions for ALL patients, results in maximal therapeutic efficacy.

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Statement of ethics: *Samples from the patients were obtained in accordance with the Helsinki Declarations. Written informed consent for genetic testing was obtained from patient and/or their parent/guardian.*

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Disclosure Summary: *The authors have nothing to disclose.*

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