

Juvenile Myelomonocytic Leukemia with *PTPN11* Mutation in a 23-Month-Old Girl

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ABSTRACT

Juvenile myelomonocytic leukemia (JMML) is a rare clonal myeloproliferative disorder affecting young children. The natural course of JMML is rapidly fatal with 80% of patients surviving less than three years. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment of JMML. We report a case of a 23-month-old girl who presented with an upper respiratory tract infection, fever, rash, diarrhea, hepatosplenomegaly and abdominal distention. Severe elevation of white blood cell count with monocytosis and myeloid progenitors in the peripheral blood was also detected. Bone marrow smear showed morphology suggestive of JMML, an unspecific immune phenotype and a normal karyotype. DNA analysis revealed a mutation in the PTPN11 gene. Therefore, the final diagnosis of JMML with somatic PTPN11 mutation was established. Following three months of cytostatic therapy with 6-mercaptopurine and low doses of cytarabine partial remission was achieved and allogeneic HSCT was successfully performed. Six months after the diagnosis, the girl was in a good condition and in a complete remission of JMML. Early diagnosis and allogeneic HSCT were crucial for successful treatment outcome.

Key words: juvenile myelomonocytic leukemia, JMML, *PTPN11* gene mutation

Introduction

Juvenile myelomonocytic leukemia (JMML) is a rare type of childhood leukemia representing 2% of hematopoietic malignancies in childhood. It is a stem cell disorder characterized by clonal hyperproliferation of monocytes and granulocytes without differentiation arrest¹. The World Health Organization has included JMML in the category of Myelodysplastic and Myeloproliferative disorders². It is characterized by young age, hepatosplenomegaly, lymphadenopathy accompanied by infiltration of other organs, particularly lungs, intestines and skin³. The natural course of JMML is rapidly fatal with 80% of patients surviving less than three years⁴. The only curative treatment modality for these children is allogeneic HSCT, with event-free survival in about half of the children⁵.

Here we report a case of 23-month-old girl diagnosed with JMML and somatic *PTPN11* mutation.

Case Report

A 23-month-old girl was admitted to our hospital due to upper respiratory tract infection accompanied with prolonged fever, rash, diarrhea and abdominal distension. Family history showed a pattern of malignancies, with the grandfather on the father's side dying from chronic lymphocytic leukemia and both grandparents on the mother's side dying from solid tumors. The mother was 37 years old at delivery. The patient is her second child after two miscarriages. The pregnancy and delivery were without complications. Birth was at term and infancy period was without any serious illnesses.

Initial physical examination did not reveal hepatosplenomegaly. But hepatosplenomegaly rapidly developed within the first week of admission, together with enlarged mesenteric and retroperitoneal lymph nodes. Initial workup revealed severe increase in white blood cell (WBC) count (up to $71.33 \times 10^9/L$) with monocytosis

(25% of WBC) and myeloid progenitors present in the peripheral blood (Figure 1a). Serological testing and DNA analysis ruled out acute Epstein-Barr virus, cytomegalovirus, human herpesvirus 6 and toxoplasma infection. Hemoglobin F was also increased (10.1%). Subsequently, bone marrow smear showed morphology suggestive of JMML: high cellularity, M:E ratio of 15:1, left shift, hyperplastic myelopoiesis with hypergranulation and moderate dysplasia, hypoplastic erythropoiesis and mild dysplastic megakaryopoiesis. Microscopic bone marrow differential count showed blasts (4%), promyelocytes (7%), myelocytes (22%), metamyelocytes (15%), bands (12%), segmented (17%), eosinophils (1%), basophils (1%), lymphocytes (7%), monocytes (8%) and erythroblasts (6%) (Figure 1b). Bone marrow analysis demon-

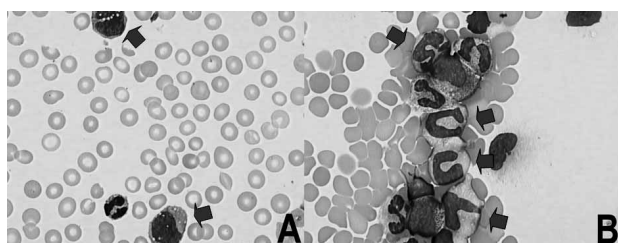


Fig. 1. Monocytes (May-Grünwald-Giemsa) in a) peripheral blood and b) the bone marrow aspirate smear.

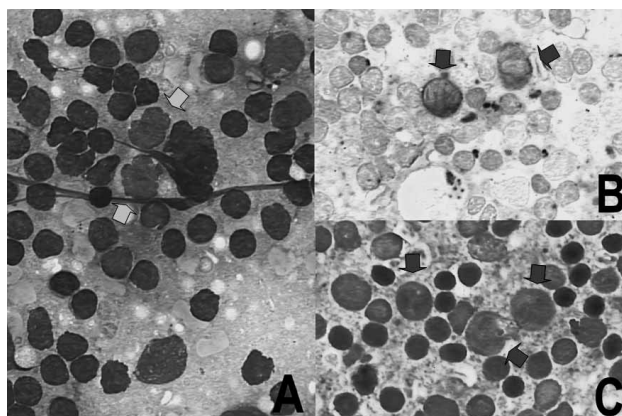


Fig. 2. The leukemic infiltrate in the fine needle aspirate of the lymph node (monocytes): a) May-Grünwald-Giemsa, b) alpha naphthyl acetate esterase and c) immunocytochemical staining with CD68.

strated an unspecific immune phenotype and a normal karyotype. The patient was negative for the Philadelphia chromosome and the *BCR/ABL* fusion gene. In further studies monocyte infiltration was detected in an endoscopic ultrasound guided biopsy of a mesenteric lymph node (Figure 2). DNA analysis revealed a mutation in the *PTPN11* gene, exon 3, G226A.

Consequently, the patient was diagnosed with JMML and allogeneic HSCT was suggested as the only curative treatment. Therefore, HLA typing of the patient and family members was performed. Her older sister was

found to be a suitable bone marrow donor. The patient was treated with three courses of cytarabine (5 days, 75 mg/m² per day, subcutaneously administered) and continuous 6-mercaptopurine (50 mg/m² per day, given orally) which resulted in normalization of WBC count and a decrease in liver, spleen and lymph nodes size. During chemotherapy she received depleted and irradiated platelet transfusions twice. Two months after the diagnosis of JMML had been established, following three courses of chemotherapy, the girl was referred to another hospital in a clinically stable condition and in a partial remission. Sequencing her buccal swab DNA did not show a mutation in *PTPN11*, exon 3, excluding germline *PTPN11* mutation. Also a new bone marrow aspiration was performed: bone marrow was hypercellular demonstrating a left-shifted trilineage hematopoiesis. Occasional megakaryocytes were found. Moderate myeloid and mild erythroid dysplasia, with M:E ratio of 1.2:1, were observed with occasional hemophagocytosis. Microscopic differential count showed blasts (8%), promyelocytes (2%), myelocytes (8%), metamyelocytes (1%), stabs (12%), segmented (17%), eosinophiles (1%), basophiles (0%), monocytes (1%), lymphocytes (13%) and erythroid progenitors (37%). This time cytogenetics showed a 3q duplication in 2/17 metaphases and a normal female karyotype in 15/17 metaphases.

JMML with somatic *PTPN11* mutation was confirmed. Preparative regimen, based on the use of busulfan (20 mg/kg given orally over 4 consecutive days), cyclophosphamide (60 mg/kg per day for 2 consecutive days) and melphalan (140 mg/m² in a single dose), preceded a family related allogeneic HSCT (done according to the EWO-MDS/EBMT protocol) which was successfully performed three months after the initial diagnosis. Six months after the diagnosis was established, the patient's condition was satisfactory and she showed no signs of JMML.

Discussion

JMML is a rare type of childhood leukemia characterized by young age, hepatosplenomegaly, lymphadenopathy accompanied by infiltration of other organs, especially lungs, intestines and skin³. Splenomegaly invariably develops rapidly in the course of this disease, creating abdominal distension with considerable discomfort. Dry cough and tachypnea are caused by lung infiltration. WBC count is mostly elevated, but in contrast to chronic myelogenous leukemia (CML) rarely exceeds 100×10⁹/L. Since prompt diagnosis is not always simple to make, morphological evaluation of the peripheral blood smear is often crucial. It demonstrates monocytosis with immature and dysplastic forms. An absolute monocyte count of ≥1000/μL is a prerequisite for the diagnosis⁶. Blasts may be present in the peripheral blood but their percentage is less than 20%³.

Two-thirds of JMML patients have the reversal to fetal red cell characteristics, including increased levels of hemoglobin F, expression of the i antigen and low car-

bonic anhydrase levels⁷. Therefore, hemoglobin electrophoresis may help establish the diagnosis. Many infectious diseases caused by Epstein-Barr virus, cytomegalovirus, human herpesvirus 6, histoplasma, mycobacteria, and toxoplasma can closely mimic JMML and further complicate the diagnostic procedure⁶.

JMML lacks the Philadelphia chromosome and the *BCR/ABL* fusion gene. Chromosomal studies of leukemic cells show monosomy 7 in about 25%, other abnormalities in 10% and a normal karyotype in 65% of patients^{3,8,9}. Myeloid progenitors also show hypersensitivity for granulocyte-macrophage colony-stimulating factor (GM-CSF)¹⁰. It is mediated by the RAS-RAF-MAP (mitogen-activated protein) kinase signaling pathway, which is pathologically activated by mutations in *RAS*, *NF1* (the gene for neurofibromatosis 1), and *PTPN11*. These mutually exclusive mutations in JMML suggest the importance of the pathological activation of RAS dependent pathways in the pathophysiology of the disease⁴. Somatic mutations in *PTPN11*, the gene encoding the protein tyrosine phosphatase SHP2, are the most frequent molecular lesions in JMML and are encountered in approximately 35% of all JMML patients¹¹. However, it is important to note that about half of children with Noonan syndrome are known to carry specific germline mutation in *PTPN11*¹² and that JMML-like myeloproliferative disorder has occurred in some of these children¹³.

The natural course of JMML is rapidly fatal. Progression to blast crisis is infrequent, but most children die from progressive respiratory and organ failure. The only curative treatment option for these children is allogeneic HSCT⁵. It is important to rule out or confirm the possibility of Noonan syndrome in newly-diagnosed children with JMML and, if a *PTPN11* mutation is present, determine whether the mutation is somatic or germline. The JMML-like disorder may spontaneously disappear in the patients with Noonan syndrome and *PTPN11* germline mutation. Therefore, reasonable therapy for these children is close observation⁴.

Despite JMML being a rare disease, it has well defined diagnostic guidelines. Splenomegaly, monocytosis ($>1 \times 10^9/L$) in the peripheral blood and less than 20% of blasts in the bone marrow are the essential clinical and hematological criteria for JMML. In patients fulfilling these criteria the diagnosis of JMML is further facilitated by the presence of one of the following: *PTPN11/RAS/NF1* mutation, clinical diagnosis of neurofibromatosis 1 (NF1) or monosomy 7. The diagnosis of JMML in patients without the *PTPN11/RAS/NF1* mutation, clinical diagnosis of NF1 or monosomy 7 should be made by employing the standard criteria. Colony assay for spontaneous growth and hypersensitivity to GM-CSF, as well as the exclusion of *BCR/ABL* rearrangement are mandatory for those patients⁶. Prognosis of the disease has been associated with three characteristic areas: sex of

the patient, age at diagnosis and other existing conditions. Males, younger than 2 and with a diagnosed Noonan syndrome have been shown to have a more encouraging prognosis. Also, low platelet count and high HbF at diagnosis have been found to be predictors of short survival^{3,8,14}.

Our patient had splenomegaly, monocytosis ($>1 \times 10^9/L$) in the peripheral blood, less than 20% of blasts in the bone marrow and a *PTPN11* mutation, which was sufficient to establish the diagnosis. The patient had no elements of Noonan syndrome phenotype which commonly includes heart malformation, short stature, learning disabilities, indentation of the chest, impaired blood clotting and facial changes¹². We also sequenced her buccal swab DNA which excluded germline mutation and JMML-like disorder. Detailed testing for a panel of infectious diseases ruled out an infectious origin of the clinical and hematologic symptoms. All those investigations were necessary prior to HSCT because JMML-like disorder can spontaneously disappear without any treatment.

It is also important to point out that standard chemotherapy, regardless of the intensity, has been proven effective only in a small number of patients⁴. Systematic evaluation of chemotherapeutic approaches in JMML is difficult due to the lack of standardized response criteria and the heterogeneity of response (i.e., clearance of leukemic cells from bone marrow but not solid organs, or *vice versa*). It appears that 6-mercaptopurine is an agent that consistently produces clinical and/or hematologic improvement, either alone or combined with cytarabine or etoposide¹⁵. In vitro 13-cis-retinoic acid has been proven to inhibit the growth of JMML cells. Hence, the COG JMML Study included it in the treatment protocol. Nevertheless, therapeutic value of 13-cis-retinoic acid for JMML still remains controversial¹⁶. Our patient responded well to combination of low doses of cytarabine and 6-mercaptopurine which resulted in a partial remission with normalization of WBC count and a decrease in liver, spleen and lymph nodes sizes. However, a cytological follow-up analysis of the bone marrow, after two months of treatment, still showed JMML with the appearance of chromosome aberration. This further contributes to the belief that the allogeneic HSCT is in fact the only curative therapy for JMML and that children diagnosed with JMML should be treated with an allogeneic bone marrow transplant as soon as a suitable donor is found⁴.

Acknowledgements

We would like to thank Professor Charlotte Marie Niemeyer from the University Clinic Freiburg for her expert advice in conducting diagnostics and enabling implementation of the *PTPN11* and *RAS* mutation analysis.

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JUVENILNA MIJELOMONOCITNA LEUKEMIJA S *PTPN11* MUTACIJOM KOD DJEVOJČICE STARE 23 MJESECA

SAŽETAK

Juvenilna mijelomonocitna leukemija (JMML) je rijetki klonalni mijeloproliferativni poremećaj od kojeg obolijevaju djeca. Prirodni tijek JMML-a brzo završava fatalno i 80% pacijenata preživi manje od tri godine. Alogena transplantacija hematopoetskih matičnih stanica jedini je kurativni tretman za JMML. Predstavljamo slučaj djevojčice stare 23 mjeseca hospitalizirane zbog infekcije gornjih dišnih putova, vrućice, osipa, proljeva, hepatosplenomegalije i distendiranog abdomena. Bila je prisutna značajno povišena razina leukocita s monocitozom i mijeloidnim progenitorima u perifernoj krvi. Analiza razmaza koštane srži pokazala je morfologiju karakterističnu za JMML, nespecifičan imunološki fenotip i normalan kariotip. DNA analizom dokazana je mutacija u *PTPN11* genu. Time je postavljena konačna dijagnoza JMML-a sa somatskom mutacijom *PTPN11* gena. Nakon tromjesečne terapije 6-merkaptopurinom i niskim dozama citarabina, provedena je alogena transplantacija hematopoetskih matičnih stanica. Šest mjeseci nakon postavljanja dijagnoze pacijentica je u dobrom stanju i kompletnoj remisiji bolesti. Rana dijagnoza i alogena transplantacija hematopoetskih matičnih stanica su od osnovne važnosti za uspješan ishod liječenja.