t(1;7;22)(p13;q21;q13) is a novel 3-way variant of t(1;22)(p13;q13) neonatal acute megakaryoblastic leukemia: A case report

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Received January 23, 2021; Accepted June 1, 2021

DOI: 10.3892/mco.2023.2614

Abstract. Acute megakaryoblastic leukemia (AMKL) is a rare disease, occurring mostly in infants and young children. The chromosomal translocation t(1;22)(p13;q13), resulting in the RBM15-MKL1 fusion gene, is a recurrent and diagnostic translocation in infants with AMKL. The present case report describes a case of a newborn girl, without Down's syndrome, with congenital AMKL. At birth, the infant had hepatosplenomegaly and the peripheral blood count revealed anemia, thrombopenia and leukocytosis, with 28% blasts. Immunophenotyping demonstrated blasts positive for CD34, CD61 and CD42b. Karyotyping of these blasts (R-banding) showed a hitherto unreported chromosomal translocation, t(1;7;22)(p13;q21;q13), a 3-way variant of the t(1;22)(p13;q13) variant. Fluorescent *in situ* hybridization analysis confirmed the presence of the RBM15-MKL1 fusion gene.

Introduction

Acute megakaryoblastic leukemia (AMKL; French-American-British M7) is a rare disease, occurring in 4 to 15% of children with acute myeloid leukemia (AML) worldwide (1-4). AMKL appears to be *de novo* in infants and in young children without Down's syndrome (DS), and patients frequently present with bone marrow fibrosis, hepatosplenomegaly and pancytopenia (5,6). The diagnosis of AMKL is based on blast cell morphology, which is suggestive of megakaryoblasts and the protein expression of platelet-associated markers (CD41, CD42b or CD61) using immunophenotyping (1,7).

The chromosomal translocation, t(1;22)(p13;q13) occurs in 10-15% of pediatric non-DS-AMKL and is a specific translocation in infants with AMKL. This translocation results in the fusion of the RNA-binding motif protein-15 (RBM15) and megakaryoblastic leukemia-1 (MKL1) genes (5,7-10). The present case report describes a case of neonatal AMKL, with a hitherto unreported 3-way translocation t(1;7;22)(p13;q21;q13).

Case report

A 31-year-old woman presented at the University Hospitals Leuven (Belgium) at 36 weeks in her second pregnancy, with a decrease in child movement for 5 days and the loss of brown fluid per vaginam for 3 days. Until then, the pregnancy was uncomplicated and no fetal abnormalities were observed.

An ultrasound examination of the fetus showed hepatosplenomegaly and a cardiotocography revealed tachycardia and small variations on the trace. An urgent cesarean section was performed, and a baby girl was born at 36 weeks and 2 days of pregnancy, with Apgar scores of 2, 6 and 7 after 1, 5 and 10 min, respectively. The patient weighed 2,700 g, measured 48 cm in length and had a head circumference of 33.5 cm. The abdomen was extremely distended due to an enlarged liver and spleen, and was hard on palpation.

Initial blood testing showed normochromic, normocytic anemia (hemoglobin, 7.3 g/dl; reference range, 14.5-22.5 g/dl), with signs of active erythropoiesis, deep thrombopenia ($39x10^9$/l; reference range, 150-$450x10^9$/l) and leukocytosis of $23.4x10^9$/l (reference range, 9.4-$34x10^9$/l) with 20% blasts, and absolute neutropenia ($2.3x10^9$/l; reference range, 5-$21x10^9$/l). The lactate dehydrogenase level was elevated to 1,403 U/l (reference range, 135-250 U/l). The cells were then analyzed using histopathology.

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Abbreviations: AMKL, acute megakaryoblastic leukemia; AML, acute myeloid leukemia; FISH, fluorescent *in situ* hybridization; MKL1, megakaryoblastic leukemia-1; RBM15, RNA-binding motif protein-15; RT-qPCR, reverse transcription-quantitative PCR

Key words: acute myeloid leukemia, chromosomal translocation, pediatric hematology

The cells were fixed with absolute methanol for 10 min, stained with May Grünwald solution for 5 min, stained with Giemsa solution for 5 min and then in buffer (pH 6.8) for 2 min, all at room temperature. Images were captured using a Leica DM LED light microscope at x500 magnification. Fig. 1 illustrates the megakaryoblasts, found in the peripheral blood smear, from May Grünwald staining, together with two normoblasts with anisopoikilocytosis of the red blood cells, a lymphocyte and irregular formed agranular platelets. The megakaryoblasts were medium sized with a high nucleus/cytoplasm ratio, a basophilic agranular cytoplasm and a round regular nucleus with fine reticular chromatin.

Immunophenotyping of the peripheral blood was performed using flow cytometry. For surface staining a 6-color protocol was used: 100 μl peripheral blood was incubated for 10 min with the following monoclonal antibodies: CD45-PerCP-Cy5.5 (20 μl; 1/2 diluted in Cell Wash; cat. no. 332784; BD Biosciences), CD61-FITC (15 μl; undiluted; cat. no. 347407; BD Biosciences), CD11b-PE (15 μl; undiluted; cat no. 333142; BD Biosciences), CD13-PE (15 μl; undiluted; cat. no. 347406; BD Biosciences), CD33-APC, (5 μl; undiluted; cat. no. 345800; BD Biosciences), CD34-FITC (15 μl; undiluted; cat. no. 345801; BD Biosciences), CD117-PE-Cy7 (5 μl; undiluted; cat. no. 339217; BD Biosciences), anti-HLA-DR-APC-H7 (5 μl; undiluted; cat. no. 641411; BD Biosciences), CD42b-PE (15 μl; undiluted; cat. no. IM1417U; Beckman Coulter, Inc.) and CD36-FITC (15 μl; undiluted; cat. no. B49201; Beckman Coulter, Inc.) at room temperature, then subsequently lysed for 10 min using 2 ml FACS-Lysing solution (BD Biosciences). For intracellular staining, a Fix and Perm reagent (ImTec Diagnostics NV) was used and monoclonal antibodies against MPO-FITC (15 μl; undiluted; cat. no. F0714; Dako; Agilent Technologies, Inc.) and CD79a-PE (15 μl; undiluted; cat. no. 333152; BD Biosciences). After staining, the samples were washed with 2 m Cell Wash (BD Biosciences) and analyzed using a FacsCanto flow cytometer (BD Biosciences) by collecting 100,000 events. For analysis, the FacsDIVA software (version 6.1.2; BD Bioscience) was used. Blast cells were gated based on their side-scatter and dim CD45 characteristics. Immunophenotyping of the peripheral blood showed a population of 28% blasts, and were positive for CD34, CD61 and CD42b and negative for cyMPO, CD13, CD117, CD33, CD36 and human leukocyte antigen-DR (Fig. 2). As the megakaryocytic markers, CD61 and CD42b were found to be positive from the peripheral blood, this suggested the patient had AMKL. Short-term culture of the peripheral blood, without mitogen, revealed a balanced translocation t(1;7;22)(p13;q21;q13), as the sole aberration in all 13 analyzed metaphases (Fig. 3A). Fluorescent *in situ* hybridization (FISH) was performed according to standard protocols and manufacturer's procedures. The commercially available probe RBM15-MKL Dual Fusion/Translocation FISH Probe (CytoTest) was used on peripheral blood metaphases from the same culture as that analyzed by conventional karyotyping. Images were captured using a fluorescence microscope (magnification, x400) equipped with an Axiophot 2 camera (Carl Zeiss AG) and a MetaSystems Isis imaging system (MetaSystems). This showed one expected fusion signal on the derivate 1 chromosome, while the second fusion was not located on the derivative 22, but on the derivative 7. This demonstrated that the observed three-way translocation was a variant of the recurrent t(1;22)(p13;q13) (Fig. 3B).

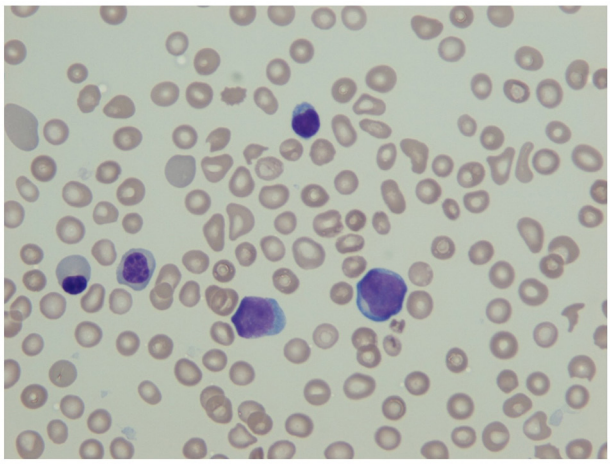

Figure 1. May-Grünwald-Giemsa staining of the peripheral blood showing 2 megakaryoblasts. In addition, 2 normoblasts with anisopoikilocytosis of the red blood cells, a lymphocyte and irregular formed agranular platelets were found. Magnification, x500.

Reverse transcription-quantitative PCR analysis of the peripheral blood showed an overexpression of ecotropic viral integration site 1 (EVI1) (data not shown). RT-PCR analysis was performed as previously described by Gröschel *et al* (11). RNA was extracted from the leukemic blast cells using a Maxwell® RSC simplyRNA Blood kit (Promega Corporation), according to the manufacturer's protocol. cDNA was subsequently synthesized using a Superscript II reverse transcriptase kit (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. EVI1 expression was quantified against the housekeeping gene, ABL1 ($^{\Delta}$Cp=Cp (MECOM)-Cp (ABL1); ratio, $2^{-\Delta Cp}$) (11). EVI1 qPCR was performed using the QuantStudio DX Real-Time PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) using 12.5 μl TaqMan Fast Advanced Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.), 2.5 μl MECOM primer probe mix, 2.5 μl ABL1 primer probe mix, 7.5 μl water and 2.5 μl cDNA (maximum, 125 ng RNA). The following primers (Integrated DNA Technologies, Inc.) were used: MECOM EVI1 forward, 5'-AGTGCCCTG GAGATGAGTTG-3', and reverse, 5'-TTTGAGGCTATCTGT GAAGTGC-3'; ABL-F-EAC forward, 5'-TGGAGATACACT CTAAGCATAACTAAAGGT-3', and reverse, 5'-GATGTA GTTGCTTGGGACCCA-3'. The following probes (IDT) were used: ABL-P_EAC-HEX, HEX-CCATTTTTGGTTTGGGCT TCACACCATT-BHQ1, and EVI1_P2 FAM-CCCCAGTGA GGTATAAAGAGGAAGAATATA-BHQ1. The following thermocycling conditions were used: 95˚C for 20 sec, 50˚C for 2 sec, at 95˚C for 1 sec and 60˚C for 20 sec (50 cycles). The SKOV3 cell line (3q26 amplified) was used as a calibrator for quantification and as a positive control, while the HL60 cell line and H_2O were used as negative controls. Positivity was defined as a sample with sigmoid amplification and a ratio (normalized to SKOV3 ratio) >0.11.

On the first day, the patient developed an intracranial hemorrhage, hypotension and renal failure. In consultation with the parents, it was decided not to start chemotherapy, as their child was critically ill. She died after 3 days.

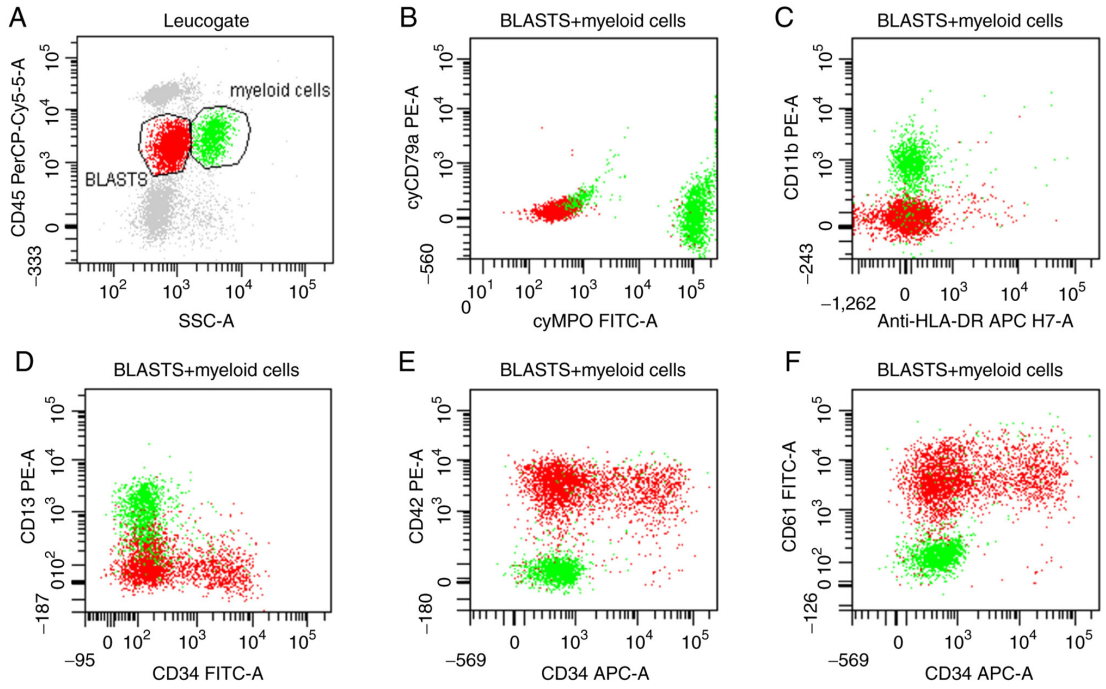

Figure 2. (A) Flow cytometric immunophenotyping of the peripheral blood. Blasts (in red) and myeloid cells (in green) are gated based on CD45/SSC characteristics. (B-D) Blasts and myeloid cells were negative and positive for cyMPO, CD11b, HLA-DR and CD13, respectively. (D-F) A subpopulation of blasts was positive for CD34, while all blasts expressed CD42 and CD61.

Discussion

AMKL is a rare subtype of AML and is predominantly found in infants (1,12). The chromosomal translocation t(1;22)(p13;q13), which results in the RMB15/MKL1 fusion gene, is specific to this subtype (5,8). Until recently, the RMB15/MKL1 fusion gene was the only recurrent genetic aberration detected in non-DS-AMKL; however, novel fusion genes have been identified over the few years, such as CBFA2R3-GLIS2 and NUP95-KDM5A (13).

The patient in the present case report presented with hepatosplenomegaly, anemia and thrombopenia, which is frequently observed in AMKL (5,6). Blood analysis revealed the diagnosis of AMKL, with the typical findings of megakaryocytes from a peripheral blood smear and was positive for CD42b and CD61 from immunophenotyping. However, several cases have been described where the diagnosis of AMKL was complicated due to bone marrow fibrosis or extramedullary disease (14-17).

Karyotyping of the blast cells showed a three-way variant of the known translocation t(1;22)(p13;q13) and FISH analysis confirmed the RBM15-MKL1 fusion gene. To the best of our knowledge, this is the first description of this translocation. A search of the Mitelman database and the literature only revealed a few variants of the translocation (1;22)(p13;q13), in addition to the novel 3-way variant t(1;7;22)(p13;q21;q13), as aforementioned. There were 2 3-way translocations described, t(1;22;14)(p13;q13;q31) and t(1;22;4)(p13;q13;q35) (3,18), as well as 3 additional 4-way translocations, t(1;22;17;18)(p13;q13;q22;q12), t(1;6;6;22)(p13;p25;q13;q13) and (t1;2;22;2)(p13;q21;q13;p23) (10,12). Whether this rare variant carries the same prognosis, is currently unclear and requires further research.

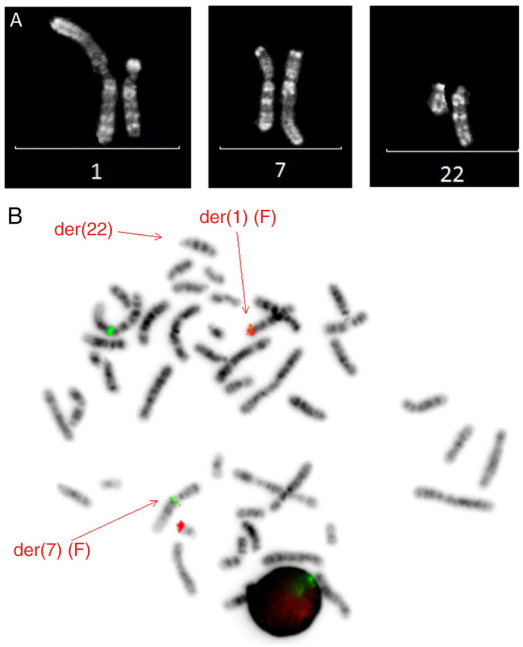

Figure 3. (A) Partial R-banded karyotype illustrating the translocation t(1;7;22)(p13;q21;q13). The normal chromosomes are shown on the left-hand side and the abnormal chromosomes are on the right-hand side. (B) Metaphase fluorescence *in situ* hybridization using the RBM15-MKL1 dual fusion translocation probe. Fusion signals were observed on the derivative chromosomes 1 and 7. A normal RBM15 (green) and MKL1 (red) signal were observed on chromosome 1 and 22, respectively. Der, derivative.

AMKL has a poor outcome, but with intensive chemotherapy regimens, an improvement in survival time has been achieved, with a reported 5-year overall survival rate of 70±6% in the AML-BMF 04 trial vs. 45±8% in the AML-BMF 98

trial (9). The decision to renounce therapy in the present case, was thoroughly discussed and was based on the comorbidities the patient had.

Acknowledgements

The authors would like to thank Ms Monique Rubens and Msc Geneviève Ameye (Department of Human Genetics, University Hospitals Leuven, Belgium) for their assistance in the preparation of the karyotyping images and FISH-analysis.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JM acquired the data from the experiments and the patient, and wrote the original draft of the manuscript. LM, NB and PV investigated the chromosomal aberrations. AU and SAJ contributed to the interpretation of the results, and made substantial contributions to conception and design. AU, LM, PV, NB and SAJ critically reviewed and edited the draft version of the manuscript. SAJ supervised the research. LM, NB and PV confirmed the authenticity of all the raw data. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Written informed consent was obtained from the parents of the patient for publication of the case report and any accompanying images.

Competing interests

The authors declare that they have no competing interests.

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