

CASE REPORT

Neonatal acute megakaryoblastic leukemia mimicking congenital neuroblastoma

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Introduction

Acute megakaryoblastic leukemia (AMKL) with t(1;22) (p13;q13) represents <1% of all acute myeloid leukemias (AML), and is associated with the *RBM15 (OTT)-MKL1 (MAL)* fusion gene. It occurs at higher incidence in the pediatric population and accounts for approximately 70% of infants with AMKL [1, 2]. In contrast to infants with 11q23-associated leukemia who frequently exhibit hyperleukocytosis, those with *RBM15-MKL1* fusion-positive AMKL usually present with decreased leukocyte count, severe anemia, and thrombocytopenia. The pathological findings show fibrosis along with the leukemic cells in the bone marrow and lymph node [3]. AMKL with t(1;22) (p13;q13) is a rare disease that is often associated with massive organomegaly. Therefore, it may be misdiagnosed as solid organ tumors including hepatoblastoma and neuroblastoma. Neuron-specific enolase (NSE), the γ -subunit of enolase, is found not only in neuroendocrine cells but also in other cells including erythrocytes and subsets of

Key Clinical Message

We describe a neonate with abdominal distension, massive hepatomegaly, and high serum neuron-specific enolase level suggestive of congenital neuroblastoma. The patient died of pulmonary hemorrhage after therapy. Autopsy revealed that the tumor cells in the liver indicated acute megakaryocytic leukemia with the *RBM15-MKL1* fusion gene.

Keywords

Acute megakaryoblastic leukemia, neuroblastoma, neuron-specific enolase, *RBM15-MKL1*.

lymphocytes [4, 5]. Increased levels of serum NSE were reported in several patients with acute lymphoblastic leukemia [6]. Here, we report on a neonate who presented with massive hepatomegaly and increased NSE levels and was misdiagnosed with congenital neuroblastoma. Autopsy revealed that the patient was finally diagnosed with *RBM15-MKL1* fusion-positive AMKL.

Material and Methods

Case report

The patient was the second daughter of healthy and non-consanguineous Japanese parents, born at 38 weeks of gestation following an uncomplicated pregnancy, and her body weight was 3164 g. At birth, she was found to have abdominal distension. Physical examination revealed hepatosplenomegaly and petechiae in the skin. Peripheral blood analysis demonstrated thrombocytopenia with a platelet count of $32 \times 10^9/L$. White blood cell count was

$17.8 \times 10^9/L$ without blastic cells. Laboratory investigations revealed the following: total bilirubin of 8.8 mg/dL, direct bilirubin of 0.7 mg/dL, lactate dehydrogenase of 2942 IU/L, aspartate aminotransferase of 248 IU/L, alanine aminotransferase of 66 IU/L, γ -glutamyl transpeptidase of 222 IU/L, blood urea nitrogen of 4 mg/dL and creatinine of 0.3 mg/dL. Serum NSE level was extremely high (1050 ng/mL; normal value <25 ng/mL) and α -feto-protein was 59,540 ng/mL (within the normal range of age-matched controls). Urinary homovanillic acid (HVA) and vanillomandelic acid (VMA) were normal. Bone marrow aspiration showed 4.8% blastic cells (Fig. 1). Immunophenotyping and karyotyping of the bone marrow sample could not be examined because of the very small size of the sample.

Abdominal MRI showed multiple nodular lesions in the liver (Fig. 2). An initial diagnosis was made of neuroblastoma stage 4S with an unknown primary lesion, although urine VMA and HVA levels were normal. She subsequently presented with massive hepatomegaly and respiratory distress. We started chemotherapy and irradiation with a diagnosis of oncologic emergency. However, she died of pulmonary hemorrhage 1 day after therapy.

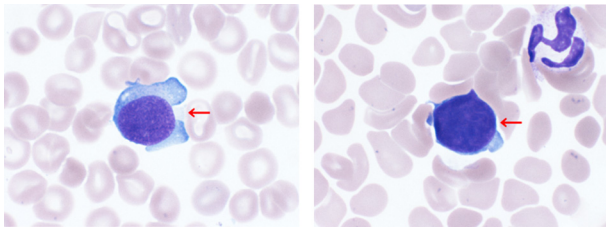


Figure 1. Bone marrow aspiration smear in the patient. Arrows indicate blast cells with large nuclei and basophilic cytoplasm (May-Giemsa staining, original magnification $\times 1000$).

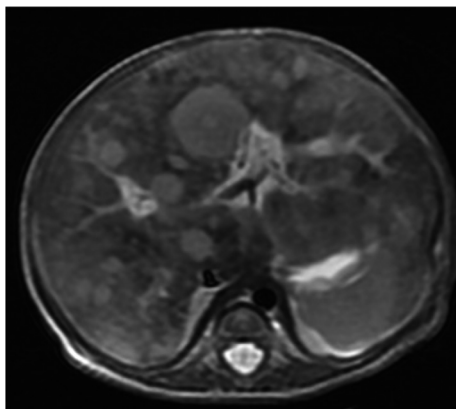


Figure 2. Abdominal MRI imaging. Multiple nodular lesions in the liver were demonstrated by fat-suppression MRI.

Molecular analysis

Reverse transcriptase-polymerase chain reaction (RT-PCR) assay for the detection of *RBM15-MKL1* fusion transcripts was performed in a not bone marrow, but liver sample with forward primer *RBM15-S* (5'-TTCCTCAGCTGCATCAGACA-3') located in *RBM15* exon 1 (GenBank accession no. NM_022768.4) and reverse primers *MKL1-AS* (5'-TCATTGAGGTCATCGGC-TAG-3') and *MKL1-AS2* (5'-TCATTGAGGTCATCGGC-TAG-3') located in *MKL1* exon 7 (GenBank accession no. NM_020831.3). The expression of β -actin was used as a control. PCR amplifications were performed using the Qiagen One-Step RT-PCR Kit (Qiagen GmbH, Hilden, Germany), in accordance with a previously published protocol [7]. Sequence analysis was directly performed on the amplified RT-PCR product by using BigDye Terminator Cycle Sequencing Chemistry (Applied Biosystems, Foster City, CA) on an automated sequencer ABI Prism 310 Genetic Analyser (Applied Biosystems), in accordance with the manufacturer's instructions.

Results and Discussion

After obtaining informed consent, autopsy was performed. At the autopsy, there were multiple nodules composed of diffuse proliferation of small round cells in the liver (Fig. 3A). Multinucleated cells with eosinophilic cytoplasm were occasionally observed. Immunohistochemically, the proliferating cells were positive for CD41, CD42b, CD43, and NSE, but negative for myeloperoxidase, TdT, CD7, CD68, CD163, PGP9.5, and tyrosine hydroxylase (Fig. 3B). The findings indicated the liver involvement of AMKL. Tumor cells were seen in the vessels of the adrenal gland and lung. Tumor cell involvement was observed in the gastric lymph node. Bone marrow showed marked myelofibrosis with scattered megakaryoblasts. Fluorescence in situ hybridization revealed chromosome 21 disomy, indicating that the patient did not have Down syndrome. Genetic analysis disclosed the *RBM15-MKL1* fusion gene specific for AMKL (Fig. 4).

Leukemia occurs much less frequently in the perinatal period than in later childhood. Although not as common as neuroblastoma, leukemia is the leading cause of death due to neoplastic disease in neonates [8]. Many cases of congenital neoplasms do not present with overwhelming signs and symptoms, and rapid and accurate diagnosis may be difficult. AMKL is a rare variant of AML, and the leukemic blasts show characteristic morphologic and phenotypic features, indicating megakaryocytoid differentiation. A distinct entity characterized by $t(1; 22)(p13;q13)$, resulting in the *RBM15-MKL1* fusion oncogene, has

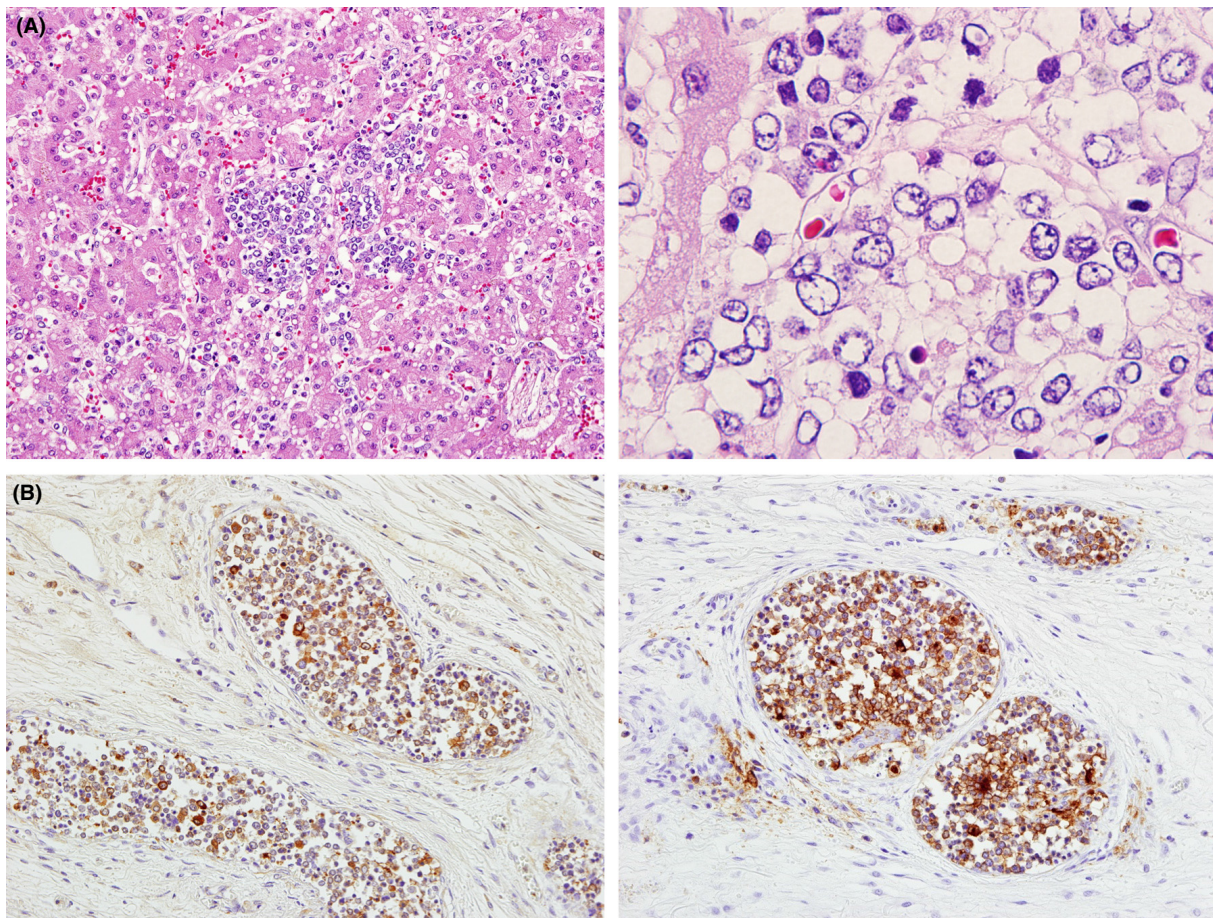


Figure 3. Histopathological findings of the liver. (A) Microscopic findings. Atypical cells are observed in the liver (hematoxylin and eosin staining – left panel: original magnification $\times 200$; right panel: original magnification $\times 1000$). (B) Immunohistochemical findings. The lesion is positive for NSE (left panel) and CD41 (right panel) (original magnification $\times 200$).

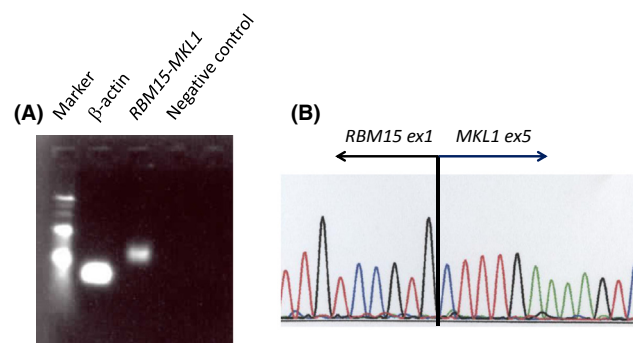


Figure 4. Molecular characterization of the *RBM15-MKL1* fusion gene. (A) RT-PCR analysis showing the presence of the *RBM15-MKL1* transcript. Lanes 1, 2 and 4 indicate a 100-bp molecular marker, an internal control, and a negative control, respectively. (B) Sequence analysis of the amplified RT-PCR product revealed an in-frame fusion between *RBM15* exon 1 and *MKL1* exon 5.

recently been identified. Of the recognized subtypes of AMKL, the disease associated with $t(1;22)$ has clinicopathologic features that present in neonates and infants

and is frequently associated with abdominal organomegaly [3, 9]. The liver was involved as the primary site in some patients with congenital leukemia [9]. Neonates with

Down syndrome sometimes present transient myeloproliferative disorder mimicking AMKL [10]; however, this patient was not diagnosed with Down syndrome.

At the initial evaluation, the patient was diagnosed with neuroblastoma stage 4S. The diagnosis of AMKL was obtained upon postmortem examination. In the present case, the elevation of serum NSE level and a few and absent blastic cells in the bone marrow and the peripheral blood might have led to a clinical misdiagnosis. NSE is usually synthesized by neurons and neuroendocrine cells, and it is a useful marker for the diagnosis and monitoring of patients with neuroendocrine tumors such as neuroblastoma and small cell lung cancer [11]. However, NSE is found not only in neuroendocrine cells but also in other cells including erythrocytes and subsets of lymphocytes [4, 5]. Some of the hematopoietic cell lines including T-cell leukemia and Epstein–Barr virus-immortalized B lymphoblastoid cell lines produce NSE. Thus, NSE is not exclusively expressed in neuroendocrine tumor cells [12].

In the present case, the most remarkable symptom was progressive hepatomegaly. Myelofibrosis is a well-recognized characteristic of AMKL. The fibrosis might have been caused by the production and secretion of transforming growth factor beta from the megakaryoblasts, which stimulates collagen synthesis in bone marrow fibroblasts [13]. There are very few documented cases of congenital leukemia in which the primary site of involvement was shown to be the liver, without initial bone marrow findings. A case of congenital anerythremic erythroleukemia was reported to present with hepatic failure and have a fetal outcome of multivisceral involvement [14]. An infant with AMKL and a complex translocation demonstrated only 5–20% blasts on the initial bone marrow aspirate and core biopsy. Therefore, myelodysplastic syndrome was initially diagnosed; however, only later did blasts become apparent in the peripheral blood [15]. In the present case, AMKL might have occurred in the fetal period because hematopoietic cells are mainly produced in the fetal liver.

Here, we report on a neonate who presented with massive hepatomegaly, increased serum NSE levels, and was misdiagnosed with congenital neuroblastoma. Autopsy revealed a final diagnosis of *RBM15-MKL1* fusion-positive AMKL. Rapid diagnosis and early treatment are required for patients with neonatal leukemia. A neonate with massive hepatomegaly would be suggestive of *RBM15-MKL1* fusion-positive AMKL.

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Conflict of Interest

None declared.

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