Clinical Case Reports

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CASE REPORT

Spontaneous remission in three cases of AML M5 with *NPM1* mutation

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Key Clinical Message

Patients with NPM1-mutated AML M5 who develop spontaneous remission (SR) after antibiotic therapy at diagnosis seem to form a favorable prognosis and chemo sensitive subtype. We report three cases of AML M5 patients with the same genotype that experienced transient SR and are now leukemia free after standard treatment.

Keywords

acute myeloid leukemia, monocytic leukemia, NPM1 mutation, spontaneous remission.

Introduction

The natural history of acute myeloid leukamia (AML) is typically unfavorable with rapid progression leading to death in the absence of specific therapy. Spontaneous Remission (SR) is a rare but well-documented event with hundreds of cases published to date. Despite reported cases and progress of conventional cytogenetic and molecular biology, the underlying mechanisms of SR remain misunderstood. Ever since the generalization and rapid start of induction chemotherapy after acute myeloid leukemia diagnosis, SR has been very rarely observed. In particular, AML with monocytic differentiation (AML M5) represents the largest subgroup of AML with spontaneous remission reported in the literature, with seven cases published since 1980 (1–7) but the existence of a link between AML M5 and SR remains elusive. For the first time, we report the cases of three patients with similar genetic characteristics diagnosed with AML M5 according to the FAB classification, who underwent rapid and transient SR.

Patients and Methods

The specimens consisted of bone marrow aspirates. AML diagnoses were established by conventional morphologic, histologic, cytochemical, immunophenotypic, and genetic criteria. Conventional cytogenetic analysis was performed on unstimulated 24-h cultures of bone marrow cells. Metaphases were obtained and analyzed using standard techniques of colchicine arrest, hypotonic treatment, and 3:1 v/v methanol/acetic acid fixation. Karyotypes were reported using the International System for Human Cytogenetic Nomenclature, based on the analysis of at least 20 metaphases when possible. DNA was extracted from bone marrow using proteinase K, followed by salt and ethanol precipitation and stored at −20°C in 10 mmol/L Tris–Cl 1 mmol/L EDTA (pH 8) buffer. PCR reactions were performed on 100 ng of DNA using *NPM1*-F (5′- TGG TTC CTT AAC CAC ATT TCT TT-3′) and *NPM1*-R (5′- TTC CAT ACA TAC TTA AAA CCA AGC A-3′) to exon 12 of *NPM1*. *CEBPA* and the exon 20 of FLT3 were also sequenced. For *CEBPA*, two fragments were amplified

using PP1-F: 5′-TCGCCATGCCGGGAGAACTCTAAC-3′; PP1-R: 5′-CTGGTAAGGGAAGAGGCCGGCCAG-3′ and PP2-F: 5′-CCGCTGGTGATCAAGCAGGA-3′; PP2-R: 5′-GGGCAAGCCTCGAGATCC-3′ primers. Only the hotspot region was sequenced for *FLT3* (FLT3-F: 5′-CCAGGAACGTGCTTGTCACCCAC-3′ and FLT3-R: 5′-TCAAAAATGCACCACAGTGAG-3′). All the PCR products were visualized on 1.5% agarose gel electrophoresis after Gel Red staining. All PCR-amplified samples were purified by standard methods and sequenced on ABI PRISM 3130 Genetic Analyzer (Life Technologies, Carlsbad, CA). The sequences were compared with those of classical databases. Informed consent was obtained from the patients for publication of this Case report.

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Patient 1

In January 1998 a 24-year-old female patient with no medical history presented with acute febrile polyarthralgia and fatigue. Physical examination showed reduced performance status (ECOG = 2) and high grade fever (40°C) but no sign of organ infiltration and no lymphadenopathy. A full-body CT scan revealed no abnormalities. Upon admission, blood cell count showed moderate pancytopenia (Hb 10 g/dL, platelets 45 G/L and WBC 2 G/L). Bone marrow aspirate was very poor and showed 58% of blast cells with monocytic appearance and lobulated nuclei. The bone marrow biopsy found a blast infiltration by cells of monocytic morphology expressing myeloperoxidase (MPO). A karyotype was not obtained, due to bone marrow aspiration difficulties. An empirical broad spectrum antibiotic therapy was started and led to prompt clinical improvement with fever defervescence, normalization of hemogram values and a complete SR documented by bone marrow examination (blasts < 5%). No colony-stimulating factors were administered and the patient was able to return home. Two months later, a hematological relapse occurred with pancytopenia. The bone marrow biopsy showed blastic infiltration comparable to that of the initial diagnosis. Cytogenetic analysis was not obtained, due to the inability to obtain mitoses. AML type chemotherapy was started and resulted in first complete remission (CR). Second leukemia relapse occurred 1 year later with hypercellular bone marrow aspirate showing 80%, MPO negative, esterase-positive monoblastic cells. Cytogenetic analysis revealed a 47XX,+8, der(14)t(1;14)(q31;q32) clone in 15/16 metaphases. Salvage chemotherapy was initiated and second CR was obtained. The patient then underwent stem cell transplant with a fully HLA-matched unrelated donor and standard myeloablative regimen. The patient is still leukemia-free 14 years after the graft. *NPM1* mutation analysis was performed on DNA extracted from a fixed cytogenetic pellet and revealed a favorable *NPM1* mutation in this patient (c.861_862insTGTC; identified in COSMIC database as COSM20861). No mutations were found in either *CEBPA* or *FLT3* (neither internal duplication (ITD) nor mutations in tyrosine kinase domain (TKD)).

Patient 2

A 33-year-old male patient with no medical history was admitted in January 2013. He had presented fever, fatigue, moderate weight loss, and anorexia for 1 month before hand. Laboratory tests showed anemia (10.2 g/dL), leukocytosis (WBC 20 G/L with 5% of blast cells), and normal platelet count (175 G/L). Bone marrow aspirate showed reduced cellularity with 10.5% of MPO positive blast cells and 14.5% of monocytes with dysgranulopoiesis. A bone marrow biopsy confirmed AML M5a diagnosis according to the FAB classification with flow cytometry revealing CD68+, CD117+, CD15+, CD34−, CD163−, and MPO− blast cells. The karyotype was normal, FISH analysis showed no *MLL* rearrangements, and molecular biology identified a type J *NPM1* mutation (an insertion of TATG, Fig. 1) without FLT3-ITD, FLT3-TKD, or *CEBPA* mutation. Due to the patient's septic condition, antibiotic therapy with piperacillin/tazobactam was introduced and cytostatic drugs were not immediately started. Blood cultures were negative and a chest CT scan revealed no abnormalities. SR was observed in the following days with normalization of Complete Blood Count (CBC) and clearance of blasts cells in the blood and bone marrow. The patient was able to return home.

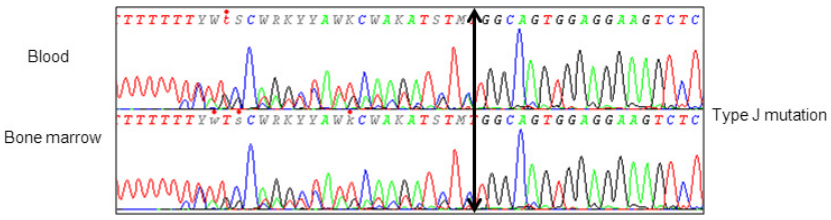

Figure 1. Electropherogram as generated by Sanger sequencing showing Type J NPM1 exon 12 mutation.

Leukemia relapse was observed 6 weeks later. Blast cell morphology, cytogenetics, and molecular biology were identical to that of first diagnosis. The patient presented severe bilateral pulmonary aspergillosis and was treated with voriconazole. The pulmonary aspergillosis has resolved and the patient is still alive in complete hematological remission 17 months after three courses of high-dose cytarabine.

Patient 3

A 74-year-old woman was hospitalized in our leukemia unit in January 2014 following the discovery of anemia and leukocytosis with monocytosis in the context of febrile pneumonia. This patient had a history of high blood pressure, diabetes, and high cholesterol. Physical examination was normal. CBC revealed a hemoglobin level of 8.8 g/dL, a white blood cell count of 21 G/L with 8% of neutrophils and 15 G/L of monocytes, and a platelet count of 350 G/L. Bone marrow aspirate displayed 14% of blasts comprising granular blasts and monoblasts, and 41% of promonocytes. The diagnosis of FAB AML M5B was established. The karyotype was normal, FISH analysis identified no *MLL* rearrangements, and molecular biology revealed a type A *NPM1* mutation (duplication of TCTG, Fig. 2) with neither FLT3-ITD nor FLT3-TKD, nor *CEBPA* mutation. Antibiotic therapy with amoxicillin/clavulanic acid was started for the pneumonia and we observed a leukemia SR with normalization of CBC and disappearance of bone marrow blasts (despite persistence of approximately 15% of dystrophic monocytes). Given the SR, the patient was allowed to return home. Leukemia relapse was observed 2 months later and bone marrow aspirate confirmed AML M5B. Flow cytometry performed on the bone marrow identified a CD13+, CD33+, CD117+, CD64+, and CD14+ blast population, in favor of a monocytic differentiation. Cytogenetics and molecular biology were identical to that of the initial diagnosis. Following AML type chemotherapy, the patient is alive, in good health and in CR.

Discussion

Spontaneous remission of acute myeloid leukemia is an extremely rare and almost always transient event, with a mean duration in the literature of 7.7 months (range 1–36) (8). Since 1979, approximately thirty cases of AML SR have been reported (8, 9), although the underlying mechanisms involved remain unclear. A potential role of bacterial and/or fungal infections and blood transfusions was suggested in SR occurrence (10) by triggering an immune and antileukemic response. Activation of the immune system and cytokines such as tumor necrosis factor (TNF), interferon gamma (IFN-γ), and interleukin-2 (IL-2) released during infections, in conjunction with an increase in the antibody-mediated cytotoxicity of Natural Killer (NK) cells and cytotoxic activity of T lymphocytes and macrophages, may play a role in the occurrence of SR (1, 2, 11, 12). Indeed, immune response might be a potential mechanism of prolonged disease control: in 2012, the case of a German patient who presented AML with a 10-year-long SR was reported. In this patient, it was demonstrated *in vitro* that NK cells displayed cytotoxic activity against the K562 myeloid leukemia cell line with upregulation of CD107a in flow cytometry (13). In addition, infection is often accompanied by a hypergammaglobulinemia, which has been associated with AML SR in some cases (1–3). The potentially beneficial effect of systemic infections in AML led to preliminary trials using bacterial extract inoculations or vaccination programs, which did not show a significant effect on survival or occurrence of remission (14, 15). Our patients did not receive any blood transfusions but all presented with infections that responded to antibiotic treatment with normalization of CBC and occurrence of SR. Unfortunately, no blood

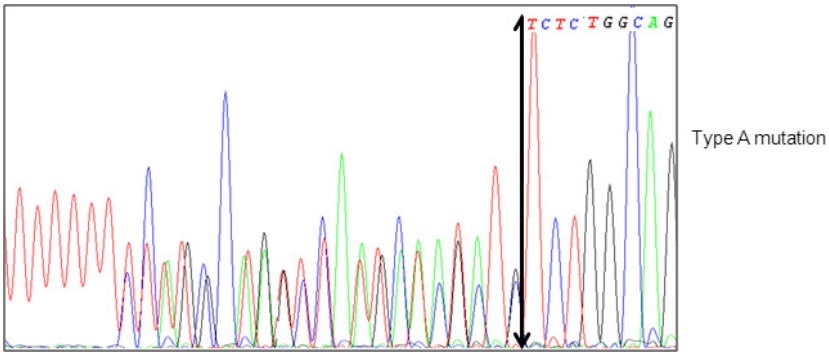

Figure 2. Electropherogram as generated by Sanger sequencing showing Type A NPM1 exon 12 mutation in DNA extracted from bone marrow.

cytokine monitoring was performed in our three patients.

To date, most cases of AML SR have been described in patients with normal karyotypes (3, 11). To our knowledge, this is the first report of three cases of SR in AML M5 according to the FAB classification with normal karyotype and *NPM1* mutations. *NPM1*-mutated AML represents a separate entity included in the AML WHO Classification since 2008, with a favorable prognosis in patients with normal karyotype AML (AML-NK) in the absence of the FLT3-ITD mutation (16). The *NPM1* mutation is the most common genetic alteration found in AML-NK and is present in approximately one-third of adult AML (17). *NPM1* mutations are located in the last exon (exon 12) and consist in duplication or insertion of small nucleotide sequences. More than 50 mutations have been reported to date, leading to a NPM1 protein variant with modification of the last seven residues, as well as four additional residues (13, 17, 18). Physiologically, *NPM1* encodes a nucleocytoplasmic shuttling protein (NPM1wt) involved in the regulation of ribosome genesis and centrosome duplication (19). *NPM1* mutation is associated with the aberrant cytoplasmic localization of NPM1, thereby disrupting its function and perturbing multiple cellular pathways (18, 20). The potential role of *NPM1* mutations in the occurrence of SR is not known but it has been previously reported that patients with AML-NK and mutated *NPM1* had a better response rate to induction chemotherapy (17, 18) which could advocate for increased chemosensitivity of blast cells with *NPM1* mutation. Recent findings suggest that *NPM1* mutations are associated with increased sensitivity to oxidative stress and that mutant NPM1 is a target of arsenic trioxide-mediated oxidative stress (21, 22). Systemic infections, usually described in cases of SR, are associated with exuberant activation of the immune system and high production of reactive oxygen species (ROS). Thus, we hypothesize that the three cases of SR presented herein might be linked by the presence of mutant NPM1, and therefore be more susceptible to oxidative stress than cases with wild-type NPM1. Moreover, we can speculate that there is a link between SR and ROS that still need to be demonstrated.

SR is an exceptional phenomenon, often transient and relatively well documented but the underlying molecular mechanisms are still unknown. *NPM1*-mutated AML M5 patients who developed spontaneous remission after antibiotic treatment of febrile neutropenia present at diagnosis seem to form a subtype with favorable prognosis and chemo sensitive disease. A better understanding of this rare process could uncover new potential therapeutic targets for acute leukemia.

Conflict of interest

None declared.

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