



Short communication

Acute promyelocytic leukemia in early pregnancy with translocation t(15;17) and variant PML/RARA fusion transcripts

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Abstract

A 32-year-old pregnant woman in the 13th gestational week was brought to Severance Hospital with gum bleeding and easy bruising. Initial laboratory results revealed anemia and thrombocytopenia. In a peripheral blood smear, 81% of leukocytes were large, abnormal promyelocytes. Bone marrow aspiration showed a hypercellular marrow with packed leukemic promyelocytes, and chromosome study revealed a karyotype of 46,XX,t(15;17)(q22;q12)[10]/46,XX[10]. In addition, variant fusion transcripts of *PML/RARA* were detected in the marrow specimen. The patient was diagnosed with acute promyelocytic leukemia (APL) and was treated with all-trans retinoic acid (ATRA) and idarubicin. One month from the patient's initial diagnosis a follow-up bone marrow examination was performed, revealing complete remission (CR). We know of no previous reports of APL during pregnancy associated with variant *PML/RARA* fusion transcripts. Here, we describe a novel case of APL in a pregnant woman with a t(15;17) translocation and variant fusion transcripts. © 2008 Elsevier Inc. All rights reserved.

1. Introduction

Although acute leukemia occurs mostly in older patients, it may also be seen in women of childbearing age, with the annual incidence of leukemia in pregnancy estimated to be 1 per 75,000–100,000 pregnancies [1–3]. Acute leukemia during pregnancy poses an immediate threat to the lives of both the mother and the fetus, making prompt diagnosis and treatment extremely important. More than 350 cases of acute leukemia during pregnancy have been reported [1,4,5]. Of these, ~50 were acute promyelocytic leukemia (APL), making APL in pregnancy a rare event [1,5–9].

Acute promyelocytic leukemia is characterized by a serious hemorrhagic syndrome (related to disseminated intravascular coagulopathy), unique morphologic findings, and response to retinoids [10]. Acute promyelocytic leukemia is related to the recurrent chromosomal abnormality

t(15;17)(q22;q12), and the rearranged gene created by this translocation encodes a chimeric protein PML/RARA that is a transcriptional repressor [11]. Briefly, three main regions of the *PML* locus are involved in the t(15;17) translocation breakpoint cluster regions (bcr): intron 6 (bcr1), exon 6 (bcr2), and intron 3 (bcr3). *RARA* breakpoints, however, always occur in intron 2, which is 17 kb in length. As a consequence, there are three possible *PML/RARA* isoforms, referred to as the long (L, or bcr1), variant (V, or bcr2), and short (S, or bcr3) forms. To our knowledge, there have been no previous reports of APL during pregnancy associated with variant *PML/RARA* fusion transcripts. Here, we describe a rare case of APL in early pregnancy with a t(15;17) translocation and variant transcripts.

2. Case report

A 32-year-old Korean woman presented in the 13th week of pregnancy after several days with gum bleeding and easy bruising. An initial complete blood count showed a hemoglobin level of 6.8 g/dL and a platelet count of 14,000/ μ L, with a white blood cell count of 19,300/ μ L. Coagulation studies revealed a prothrombin time of 18.1

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seconds (reference range, 9.8–13.4), an activated partial thromboplastin time of 26.0 seconds (reference range, 27.9–41.6), a fibrinogen level of 65 mg/dL (reference range, 200–400) and a D-dimer level of 9,347 ng/mL (reference range, 0–243). In the peripheral blood smear, 81% of the white blood cells were large, abnormal promyelocytes showing fine or heavy cytoplasmic granules, with an occasional folded nucleus. The patient's bone marrow was markedly hypercellular and replaced by many large abnormal promyelocytes with heavy cytoplasmic granules, and occasional Faggot cells (Fig. 1). Flow cytometry showed the blasts to be positive for CD13, CD33, and CD45, and negative for CD3, CD7, CD10, CD14, CD19, CD20, CD22, CD52, CD79a, CD117, p-glycoprotein and TdT.

The patient was diagnosed with APL during the first trimester and terminated her pregnancy by medical decision. Thereafter, induction chemotherapy was performed according to the modified AIDA protocol [12]. Follow-up bone marrow biopsy specimens obtained after 2 weeks still displayed 29.4% leukemic promyelocytes in all nucleated cells; however, at 1 month after the patient's initial diagnosis a third bone marrow examination was performed, revealing complete remission in her marrow.

3. Molecular study

Multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) assay by dual-priming oligonucleotides was performed with a Seeplex leukemia *PML/RARA* kit (Seegene, Seoul, Korea), which is designed to detect the long, short, and variant forms of *PML/RARA* fusion transcript. The dual-priming oligonucleotide method was as previously described [13–15]. Cycling conditions were as follows: 94°C for 15 minutes (1 cycle); 94°C for 30 seconds, 60°C for 1 minute 30 seconds, 72°C for 1 minute 30 seconds (37 cycles); and 72°C for 10 minutes (1 cycle).

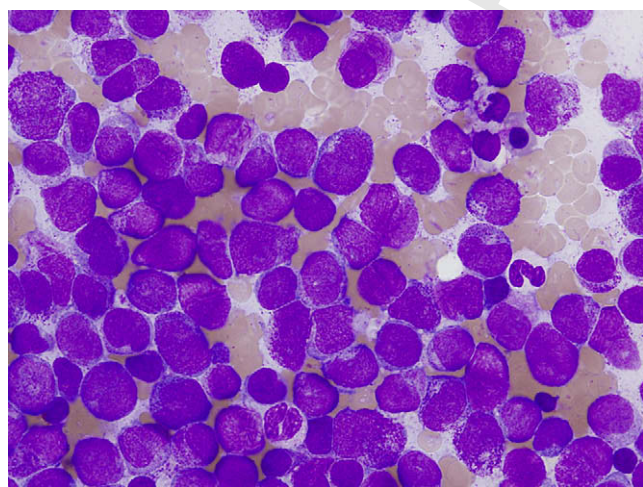


Fig. 1. Bone marrow smear (Wright–Giemsa stain, $\times 1,000$). The patient's bone marrow was markedly hypercellular and replaced by many large abnormal promyelocytes with heavy cytoplasmic granules.

The PCR products were analyzed by 2% agarose gel electrophoresis at 100 V for 25 minutes.

The purified PCR product was inserted into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA) according to the manufacturer's specifications. Plasmid DNA was prepared using a plasmid mini kit (Qiagen, Hilden, Germany). Sequencing was performed using a BigDye terminator v. 3.1 cycle sequencing kit (Applied Biosystems [ABI], Foster City, CA) and the M13F/M13R primer on a DNA sequencer (ABI model 310). Sequence analysis was done using the Ensembl database (<http://www.ensembl.org/index.html>).

4. Results

With 20 cells analyzed, we found the karyotype of the patient to be 46,XX,t(15;17)(q22;q12)[10]/46,XX[10]. Fluorescence in situ hybridization (FISH) signals from the *AML1/ETO*, *CBFB/MYH11*, *MLL*, and *BCR/ABL* probes (Vysis; Abbott Molecular, Des Plaines, IL) were within reference ranges, whereas the *PML/RARA* FISH gave a result of nuc ish(PML $\times 3$),(RARA $\times 3$),(PML con RARA $\times 2$)[250/310], consistent with the typical signal patterns of APL, in 81% of the nuclei examined. The RT-PCR analysis for *PML/RARA* rearrangement showed two separate bands of a suspected *bcr2* variant (Fig. 2). The PCR products of sizes 719 and 574 bp (145 difference) were amplified from the bone marrow specimen, and a search of the Ensembl database revealed partial deletion (147 bp) of exon 6 of *PML* (ENSG00000140464) in the two cloned PCR products, which were then compared with the typical L-form fusion transcript. By cloning and sequencing, we discovered that these two bands were isoforms in which one exon (exon 5; 144 bp size) of the *PML* gene was spliced out of the smaller products (minor PCR products), and one sequence deletion (A) of *RARA* exon 3 generated a frameshift and a premature stop codon (TGA) in the *RARA* (ENSG00000131759) coding region of the smaller fusion transcript.

5. Discussion

Hematologic malignancies associated with pregnancy have been reported in the literature, such as Hodgkin disease, acute leukemia, non-Hodgkin lymphoma, myelodysplastic syndrome, and, less frequently, essential thrombocythemia, chronic myeloid leukemia, chronic lymphocytic leukemia, and mycosis fungoides [16], although their occurrence is uncommon. Among these, acute myeloid leukemia accounts for more than two-thirds of leukemias during pregnancy, with the diagnosis generally made during the second or third trimesters [1].

An even rarer event is APL in pregnancy, with <60 cases in the literature [5,8]. Acute promyelocytic leukemia, which represents ~10% of all cases of acute myeloid leukemia, is characterized by a clonal expansion of immature myeloid precursors in the bone marrow that are arrested at the promyelocytic stage of development [17]. Reciprocal

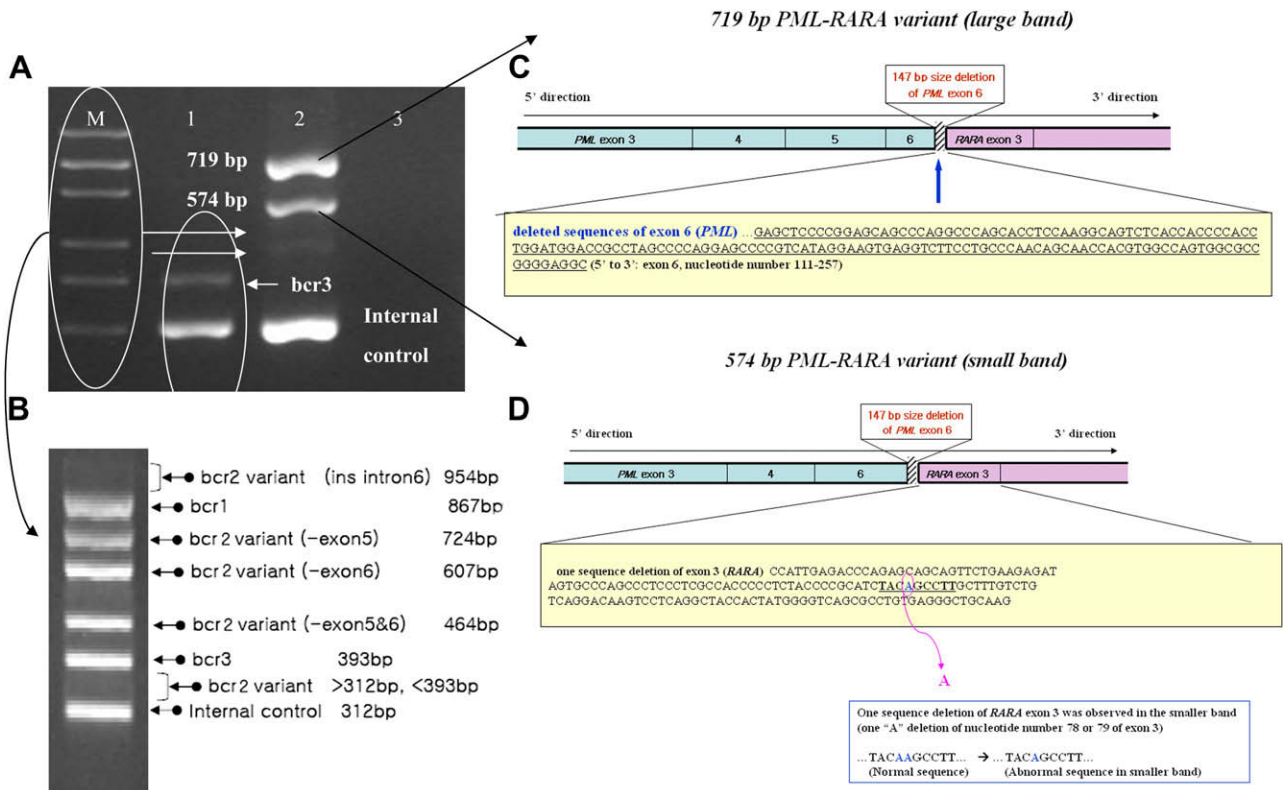


Fig. 2. Detection of alternatively spliced fusion transcript variants by reverse transcriptase-polymerase chain reaction (RT-PCR). (A) PCR products of sizes 719 and 574 bp were amplified from bone marrow specimens at initial diagnosis. Lane M, size markers for *PML/RARA* fusion transcripts; lane 1, positive control for short form (bcr-3) PCR product; lane 2, variant *PML/RARA* PCR products of the present study. (B) Size markers for various *PML/RARA* fusion transcripts (supplied by Seegene company, Korea; <http://www.seegene.com>). (C) Diagrammatic representation of the larger *PML/RARA* fusion transcript (719 bp) in this patient, with detailed sequencing information for aberrantly deleted sequences. (D) Diagrammatic representation of the smaller *PML/RARA* fusion transcript (574 bp) in this patient. One sequence deletion (A) of *RARA* exon 3 generated a frameshift and a premature stop codon (TGA) in the *RARA* coding region of the smaller fusion transcript.

translocation involving chromosomes 15 and 17, t(15;17)(q22;q12), is a characteristic feature of APL, and the rearranged gene created by this translocation encodes a chimeric protein PML/RARA that is a transcriptional repressor [11]. Breakpoints in the *RARA* gene consistently occur in its second intron (although we have encountered one exception; unpublished data [18], whereas breakpoints in *PML* introns 6 and 3 and within exon 6 result in three distinct *PML/RARA* fusion transcripts commonly referred to as the L (long, bcr1), S (short, bcr3), and V (variable, bcr2) isoforms, respectively.

Although a bcr2 transcript is the most rare type, several studies have reported the bcr2 frequency to be ~5% (reported range, 2.3–11%) in patients with APL [18,19]. This low frequency, in our opinion, explains why variant *PML/RARA* fusion transcripts have rarely been detected in APL cases during pregnancy—itsself a rare event. Although some are controversial, several reports address prognostic relevance for the 3 common transcripts (bcr1, bcr2, and bcr3), in addition to other rare variants (e.g., [20]). Among these, an association between the insertion of exon 7a of the *PML* gene and the aggressive nature of APL has been well documented by some authors, with such novel fusion

transcripts (exon 7a insertion) reported in at least three APL cases [21,22], as well as one case in our experience (unpublished data). Notably, all the patients were dead within 16 days of the initial APL diagnosis. Due to the rarity of APL during pregnancy, the biological relevance of atypical transcripts still remains unclear.

On the basis of this and our previous report [7], however, we suggest that the most important prognostic factor for pregnancies complicated by APL is not the type of fusion transcript or any additional chromosomal abnormalities, but instead prompt differential diagnosis and treatment. To avoid a diagnostic delay, a peripheral blood smear including a differential count should be performed when thrombocytopenia or a disseminated intravascular coagulopathy-like syndrome is observed during pregnancy, as in the present case.

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