



Case Report

Emergence of RUNX1-RUNX1T1 in a case of PML-RARA positive Acute Promyelocytic Leukemia: A rare case of dual translocation identified by RT-PCR

Sarita Kumari¹, Amit Kumar¹, Khurshid Mallick¹, Avinash Upadhyay¹

¹Department of Pathology, Mahavir Cancer Sansthan and Research Centre, Patna, India

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ABSTRACT

We report a 58-year female diagnosed with acute promyelocytic leukemia, initially PML-RARA positive by RT-PCR. Following initiation of all-trans retinoic acid-based therapy, the emergence of RUNX1-RUNX1T1 (t (8;21)) was detected in peripheral blood and bone marrow. Molecular studies via nested and real-time PCR confirmed dual translocation, with a bcr1 variant of PML-RARA and 260 bp AML1-ETO fusion product. This case highlights the importance of comprehensive molecular surveillance during acute promyelocytic leukemia treatment to detect early clonal evolution, which may affect prognosis and therapeutic decisions.

Correspondence:

Sarita Kumari

Scientist, Department of Pathology

Mahavir Cancer Sansthan and Research Centre, Patna, India

ORCID ID: 0009-0000-9947-9821

Email: saritadipti@gmail.com



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INTRODUCTION

Acute promyelocytic leukemia (APL) is a distinct variant of acute myeloid leukemia (AML) defined by the translocation t (15;17) (q24; q21), leading to the PML-RARA fusion gene. This gene disrupts retinoic acid signaling and causes differentiation arrest of promyelocytes. The use of all-trans retinoic acid (ATRA) with arsenic trioxide (ATO) or anthracycline chemotherapy has transformed APL into a highly curable malignancy.^{1,2}

Despite high cure rates, clonal evolution may occur under treatment pressure. Although t(8;21)(q22;q22), resulting

in the RUNX1-RUNX1T1 fusion, is typically associated with AML-M2, its emergence in APL cases has been rarely reported and is often linked to secondary clonal evolution.³ This phenomenon may reflect genomic instability or expansion of a pre-existing subclone.⁴

Molecular tools such as nested and real-time RT-PCR now enable sensitive detection of such fusion transcripts in peripheral blood, aiding in minimal residual disease (MRD) monitoring and early identification of clonal shifts.⁵

We report a rare case of a 58-year woman with classical PML-RARA-positive APL, who was later found to harbor a RUNX1-RUNX1T1 fusion gene after induction therapy. This dual translocation was detected by RT-PCR from bone marrow and peripheral blood, suggesting therapy-associated emergence of a new leukemic clone.

CASE REPORT

In January 2023, a 58-year female presented to the Department of Pathology at Mahavir Cancer Sansthan and Research Centre, Patna, with complaints of generalized weakness and fatigue. Her complete blood count revealed hemoglobin of 6.9 g/dL, a leukocyte count of $14.96 \times 10^9/L$ with approximately 40% blast cells on peripheral smear, and a platelet count of 1.2 lakh/ μL . Bone marrow aspiration was markedly hypercellular, showing 50% atypical promyelocytes with prominent azurophilic granules and Auer rods. Cytochemical staining confirmed strong myeloperoxidase positivity in the blasts.

Immunophenotyping by flow cytometry on bone marrow aspirate revealed a typical APL profile with expression of CD45⁺, CD33⁺ (bright), CD13⁺ (heterogeneous), CD117⁺ (dim to moderate), CD64 (dim), and cyMPO⁺, with absence of CD34 and HLA-DR consistent with APL (FAB M3).

Peripheral blood samples were collected at presentation. Total RNA was isolated from mononuclear cells using TRIzol reagent (Invitrogen, USA), and RNA integrity was confirmed by Nanodrop spectrophotometry. One microgram of RNA was reverse transcribed using SuperScript III (Thermo Fisher Scientific) and random hexamers.

Fusion transcript detection was performed using nested RT-PCR targeting both PML-RARA and RUNX1-RUNX1T1 transcripts with primers from van Dongen et al.⁶ Primary PCR used:

PML-RARA: PML-A1 (5'-GGA AAG GAC CAA GGA CTT ACG AGA A-3') and RARA-B (5'-CAG CTC GTT CCT CTT CCA GCT G-3')

RUNX1-RUNX1T1: AML1-A (5'-TAA TAC CAG TGC CAG TGG TGG TAA C-3') and ETO-B (5'-TTC TCC TCT CCA GTT TGC TTC TGA-3')

Nested PCR used internal primers: PML-C1/RARA-D and AML1-C/ETO-D. Each 20 μL PCR reaction contained 2.0 mM MgCl₂, 0.2 mM dNTPs, 0.2 μM primers, and 0.3 U Taq DNA polymerase. PCR cycling was performed on a Veriti™ Thermal Cycler with standard thermal conditions.

Products were run on 2% agarose gel stained with ethidium bromide, using a 100 bp DNA ladder. Each run included positive (PML-RARA and AML1-ETO-positive) and negative (no template) controls.

For quantitative confirmation, real-time PCR (qPCR) was performed with the TRUPCR PML-RARA Quantitative RT-PCR Kit (3B BlackBio Biotech India Ltd.) targeting the bcr1 variant. Reactions were run on the ABI 7500 Real-Time PCR System using the manufacturer's protocol. Ct values were interpreted using a five-point standard curve, and a 214 bp product confirmed the bcr1 breakpoint.

Molecular analysis using nested RT-PCR and real-time quantitative PCR (qPCR) confirmed the presence of dual fusion transcripts AML1-ETO (RUNX1-RUNX1T1) and PML-RARA in the peripheral blood samples of the patient.

As depicted in Figure 1, nested RT-PCR yielded two distinct amplification products. A 260 base pair (bp) band was observed in Lane 2, corresponding to the AML1-ETO fusion transcript, indicative of a t (8;21) translocation. Simultaneously, a 214 bp band was evident in Lane 4, consistent with the bcr1 variant of the PML-RARA fusion gene, associated with the classical t(15;17) translocation seen in acute promyelocytic leukemia.

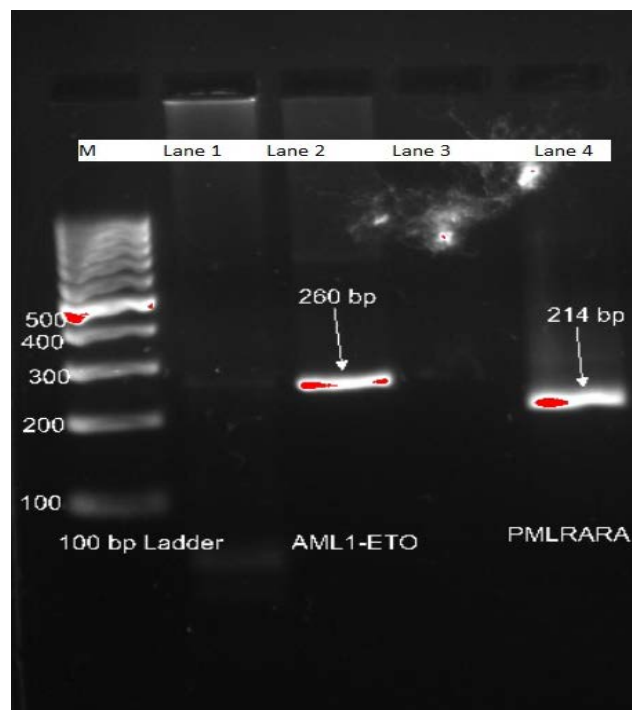


Figure 1: Gel electrophoresis showing the presence of two distinct fusion transcripts

Lane M: 100 bp molecular weight ladder with defined bands at 100, 200, 300, 400, and 500 bp

Lane 1: Negative control (H₂O), demonstrating absence of contamination

Lane 2: Positive band at 260 bp for AML1-ETO

Lane 4: Positive band at 214 bp for PML-RARA (bcr1 breakpoint)

Lane 3: Smearing artifact observed, likely due to primer-dimer or nonspecific product formation

These results confirm the simultaneous presence of two distinct fusion transcripts in the same patient sample, supporting the hypothesis of clonal evolution or coexistence of leukemic subclones (fig. 1).

DISCUSSION

Acute promyelocytic leukemia, a distinct subtype of acute myeloid leukemia, is characterized by the translocation $t(15;17)(q24;q21)$, resulting in the PML-RARA fusion gene. This fusion blocks myeloid differentiation, which is effectively reversed using all-trans retinoic acid with

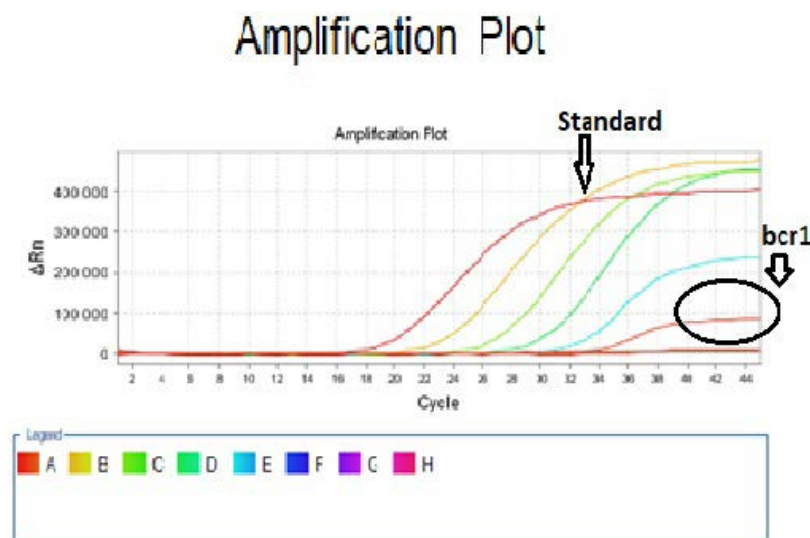


Figure 2: qPCR Amplification Plot

Real-time PCR analysis targeting the PML-RARA *bcr1* variant further validated the nested PCR findings. The amplification plot displayed a sigmoidal curve for the patient sample (black-circled curve), which crosses the threshold cycle (Ct) in alignment with the standard curve, thereby confirming quantifiable expression of the PML-RARA fusion transcript. (fig. 2)

The qPCR was performed using a five-point standard curve with known copy numbers (10^5 to 10^1). The patient's sample exhibited amplification within this dynamic range, indicating that the detected transcript level falls within reliable quantitation limits. The amplification efficiency was consistent across replicates, demonstrating high assay sensitivity and specificity.

The concurrent detection of PML-RARA and RUNX1-RUNX1T1 fusion transcripts is an uncommon finding and may indicate the coexistence of two genetically distinct leukemic clones or the clonal evolution of a single malignant population during the course of therapy. Such dual translocations could arise as a result of genomic instability or selective pressure induced by treatment, reflecting a dynamic leukemic landscape. In this case, the patient was initiated on ATRA-based induction chemotherapy, the standard regimen for acute promyelocytic leukemia.

However, given the unexpected emergence of a $t(8;21)$ -associated clone, close serial molecular monitoring was advised to assess treatment response, evaluate evolving clonal architecture, and allow for timely therapeutic modifications should the RUNX1-RUNX1T1-positive clone gain clinical predominance.

arsenic trioxide or chemotherapy. Prognosis has improved significantly, with survival rates exceeding 90% in low-risk cases.^{1,2}

However, additional chromosomal abnormalities during or after treatment can complicate disease management. One notable example is the $t(8;21)(q22;q22)$ translocation, which produces the RUNX1-RUNX1T1 fusion gene, typically associated with AML-M2 and a distinct prognosis.³

In our case, the patient showed classical features of APL, confirmed by morphology, immunophenotyping, and detection of PML-RARA (*bcr1* variant) via nested and real-time RT-PCR. Unexpectedly, a concurrent RUNX1-RUNX1T1 fusion transcript was also identified, suggesting the presence of a second translocation, $t(8;21)$. This rare finding in APL has been sporadically reported. Neto et al. described a similar case where $t(8;21)$ appeared during APL therapy, likely from a pre-existing subclone expanding under treatment pressure.⁴

Two main hypotheses may explain this: (1) coexistence of distinct leukemic clones at diagnosis, or (2) therapy-driven clonal evolution, acquiring new cytogenetic abnormalities. The latter aligns with evidence of genetic heterogeneity in AML and therapy-induced clonal shifts.⁷ This underscores the importance of molecular surveillance, not just for minimal residual disease (MRD) but also for tracking emergent genetic events.⁵

Clinically, the emergence of RUNX1-RUNX1T1 in APL introduces new challenges. While both PML-RARA and RUNX1-RUNX1T1 are sensitive to cytarabine, their coexistence could suggest resistance, relapse risk, or the need to modify therapy. It remains uncertain whether

ATRA-ATO alone is adequate, or if regimens targeting the t(8;21) clone are necessary.

This case illustrates the value of combining nested and real-time RT-PCR for detailed molecular profiling. RT-PCR's sensitivity enables detection of cryptic or emergent clones from peripheral blood, supporting real-time disease monitoring. Nonetheless, limitations exist. As a single case, it lacks generalizability, and longitudinal clonal evolution data are not yet available. Moreover, without concurrent cytogenetic studies (e.g., FISH, karyotyping), it remains unclear whether both translocations reside in the same clone or represent separate populations.

CONCLUSION

This case highlights a rare instance of co-detection of PML-RARA and RUNX1-RUNX1T1 fusion transcripts in a patient diagnosed with acute promyelocytic leukemia, suggesting either clonal heterogeneity or evolution. It reinforces the critical role of comprehensive molecular diagnostics, particularly nested RT-PCR and quantitative qPCR, in uncovering hidden or emergent chromosomal abnormalities that may alter disease prognosis or management.

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Conflict of interest: None.

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