



Cytogenetically cryptic PML::RARA fusion in acute promyelocytic leukemia: Testing strategies in the modern era

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ABSTRACT

Acute promyelocytic leukemia (APL) is a unique leukemia that is characterized by the *PML::RARA* fusion. This fusion is often detected by conventional karyotype and fluorescence in situ hybridization (FISH); however, rare cases are cryptic and require molecular techniques to identify the *PML::RARA* fusion. Furthermore, as the incidence of these cases is rare, analysis by a targeted next-generation sequencing (NGS) panel of myeloid associated genes has never been reported. Herein, a clinical APL case is reported where the *PML::RARA* fusion was detected only by reverse transcriptase-polymerase chain reaction (RT-PCR), thus underscoring the necessity of utilizing complementary techniques when suspicion for APL is present.

1. Introduction

Acute promyelocytic leukemia (APL) is a unique acute myeloid leukemia (AML) that is clinically a medical emergency and requires a rapid diagnosis. As conventional induction chemotherapy is ineffective and potentially fatal, initiation of all-*trans* retinoic acid (ATRA) is often advised when any suspicion for APL is present. As such, it is imperative to provide a quick and reliable result of the t(15;17) resulting in a *PML::RARA* fusion, either by fluorescence in situ hybridization (FISH) or reverse transcriptase-polymerase chain reaction (RT-PCR). Herein, we describe a case of APL which had clinical, morphologic, and immunophenotypic features compatible with APL despite FISH being negative for the *PML::RARA* fusion. Fortunately, RT-PCR revealed the presence of the *PML::RARA* fusion allowing for appropriate therapeutic decision making.

2. Case

A 54 year old male presented to the emergency department (ED) at an outside hospital (OSH) with left leg swelling and was found to have a left femoral vein thrombosis. He was subsequently prescribed rivaroxaban; however, his complete blood count (CBC) revealed a white blood count (WBC) of $2.8 \times 10^9/L$, hemoglobin (hgb) of 13.8 g/dL, and platelets (plt) $224 \times 10^9/L$. Two months later, he presented again to the ED at an OSH due to a syncopal episode and again, the CBC revealed

WBC $1.64 \times 10^9/L$, hgb 11.7 g/dL, and plt $94 \times 10^9/L$. One month later, a bone marrow biopsy was performed at an OSH with suspicion for an acute leukemia. At this time, the patient was transferred to our institution and a repeat bone marrow biopsy was performed for an expedited evaluation. The CBC at the time of biopsy revealed WBC $1.6 \times 10^9/L$, hgb 9.4 g/dL, and plt $69 \times 10^9/L$. The peripheral blood film revealed pancytopenia without identifiable promyelocytes or cells with Auer rods. The bone marrow aspirate smear revealed numerous promyelocytes comprising 77% of a 300 cell differential. The promyelocytes contained numerous primary granules as well as occasional cells with many thick Auer rods. Additionally, the nuclear features included those that are bilobed inducing a “butterfly” or “hourglass” appearance (Fig. 1a). The bone marrow trephine biopsy revealed significantly left-shifted maturation primarily composed of promyelocytes. By flow cytometric analysis, the neoplastic promyelocytes demonstrated high side scatter vs. CD45 gating. The neoplastic promyelocytes expressed cytoplasmic myeloperoxidase and CD117 while being negative for CD34 and HLA-DR (Fig. 1b & 1c). By immunohistochemistry, the promyelocytes are positive for CD117 and myeloperoxidase and negative for CD34. a. Bone marrow aspirate demonstrating abnormal promyelocytes, some of which contain frequent thick Auer rods. b, c. Multiparametric flow cytometric findings indicating a high side scatter, positivity for CD117, and absence of CD34 and HLA-DR expression.

Genetic studies were performed and by FISH analysis, the *PML::RARA* dual color dual fusion probe set (Abbott Diagnostics, Abbott Park,

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IL, USA) (Fig. 2a) and *RARA* dual color break apart probe set (Abbott) (Fig. 2b) were negative for the *PML::RARA* fusion as well as a *RARA* rearrangement. Karyotype analysis revealed a normal 46,XY[20] male karyotype (Fig. 2c). RT-PCR was concurrently performed and revealed the long fusion transcript (bcr1) of *PML::RARA* associated with t(15;17) (q24;q21) at a transcript level of 787.739 with *ABL1* as the control gene (Quest Diagnostics, Chantilly, VA, USA). Lastly, a targeted next-generation sequencing (NGS) panel of 65 myeloid associated genes (SmartGenomics Myeloid Profile, PathGroup, Nashville, TN, USA) was performed and revealed a *FLT3* p.D835Y variant. a. *PML::RARA* dual fusion, dual color probe set demonstrating a lack of *PML::RARA* fusion. b. *RARA* break apart probe set demonstrating a lack of *RARA* rearrangement. c. Karyogram demonstrating a normal male karyotype in 20 metaphase cells.

Prior to reaching the diagnosis of APL, the patient was initially started on ATRA 60 mg BID and upon reaching a definitive molecular diagnosis of APL, arsenic trioxide (ATO) was initiated. Day 28 bone marrow revealed morphologic remission and RT-PCR transcript level was 6.572. He eventually completed cycle 1 of consolidation with ATRA and ATO with an undetectable transcript level in the peripheral blood.

3. Discussion

APL is a subtype of AML that is defined by the t(15;17)(q24;q21) in greater than 98% of cases, resulting in the *PML::RARA* fusion transcript that promotes leukemogenesis [1]. By conventional cytogenetic methods, the *PML::RARA* fusion is detected in 70–90% of patients.

Nonetheless, there are limitations observed in conventional karyotyping, such as poor banding, complex chromosomal rearrangements, or even a cryptic insertion of *PML* into the *RARA* gene [2]. As such, complementary techniques are necessary for the accurate and timely detection of the *PML::RARA* fusion, including FISH, RT-PCR, or sequencing.

The present case serves as an illustration of how alternative techniques, coupled with the high clinical and pathologic suspicion, yielded the correct diagnosis. During the diagnostic evaluation for APL, the utilization of FISH is common; however, limitations exist due to different probe sizes available. Brockman et al. previously described the challenges of identifying cryptic *PML::RARA* fusions by utilizing FISH [3]. In their series of 38 APL cases, two cases were shown to have a cryptic rearrangement by alternate FISH probes, thus illustrating the importance knowing the pitfalls of the utilized *PML::RARA* dual fusion probes as well as *RARA* break apart probe set. This workflow is often utilized at the authors' institution, which will commonly use the *PML::RARA* dual fusion probe set while rarely employing the *RARA* break apart probe set in cases that are suspicious for APL. Additionally, as RT-PCR is routinely performed for *PML::RARA* concurrently with FISH, which invariably has a shorter turnaround time in our experience, this is the first case of cytogenetically cryptic *PML::RARA* in our lab. In the modern era of genetic stewardship, this method has proven most effective as illustrated by the extreme rarity of this event and the ability to still identify the *PML::RARA* fusion efficiently using our workflow.

Next, in the rare instances in which the *PML::RARA* fusion cannot be detected by karyotypic and FISH analysis, there have been multiple

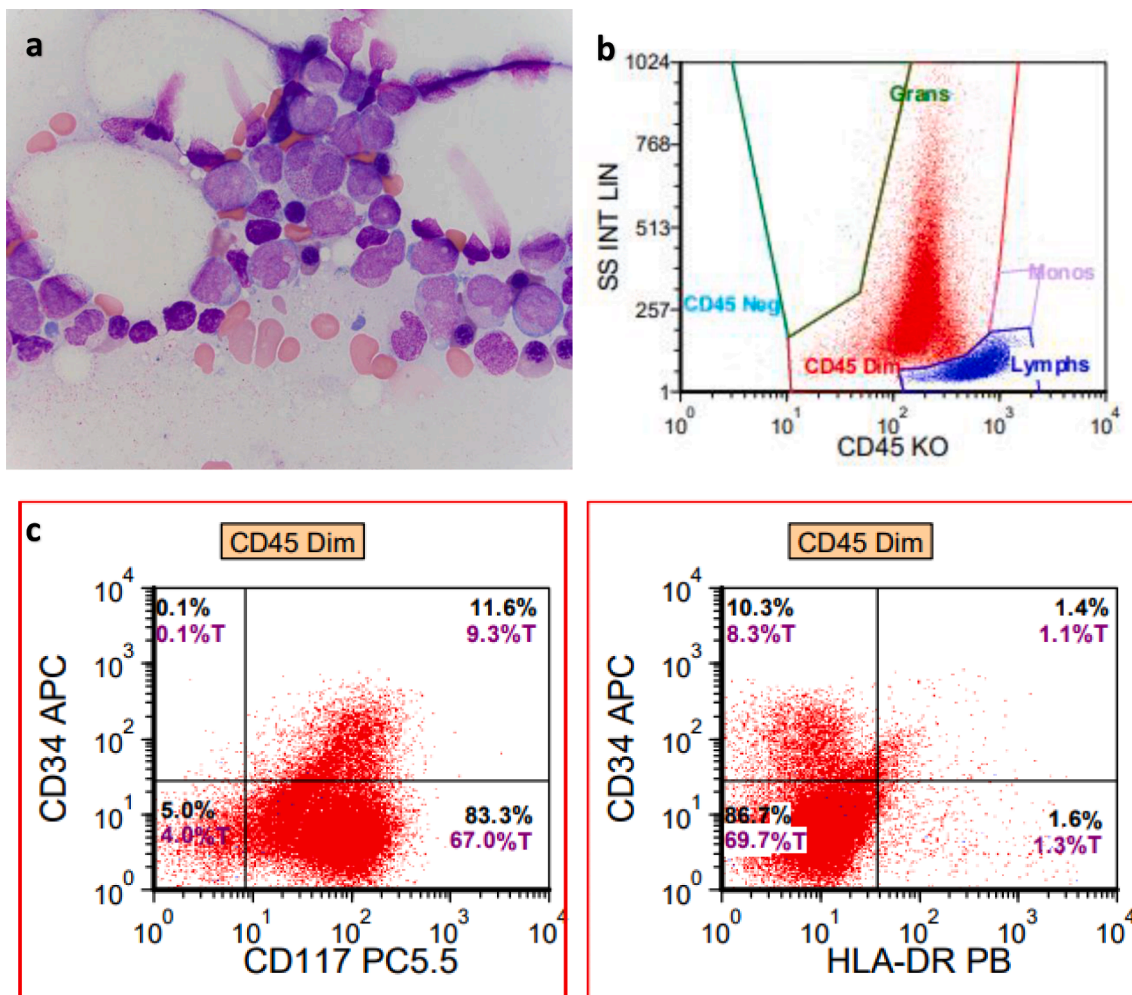


Fig. 1. Morphologic and immunophenotypic findings of APL with cryptic *PML::RARA* fusion.

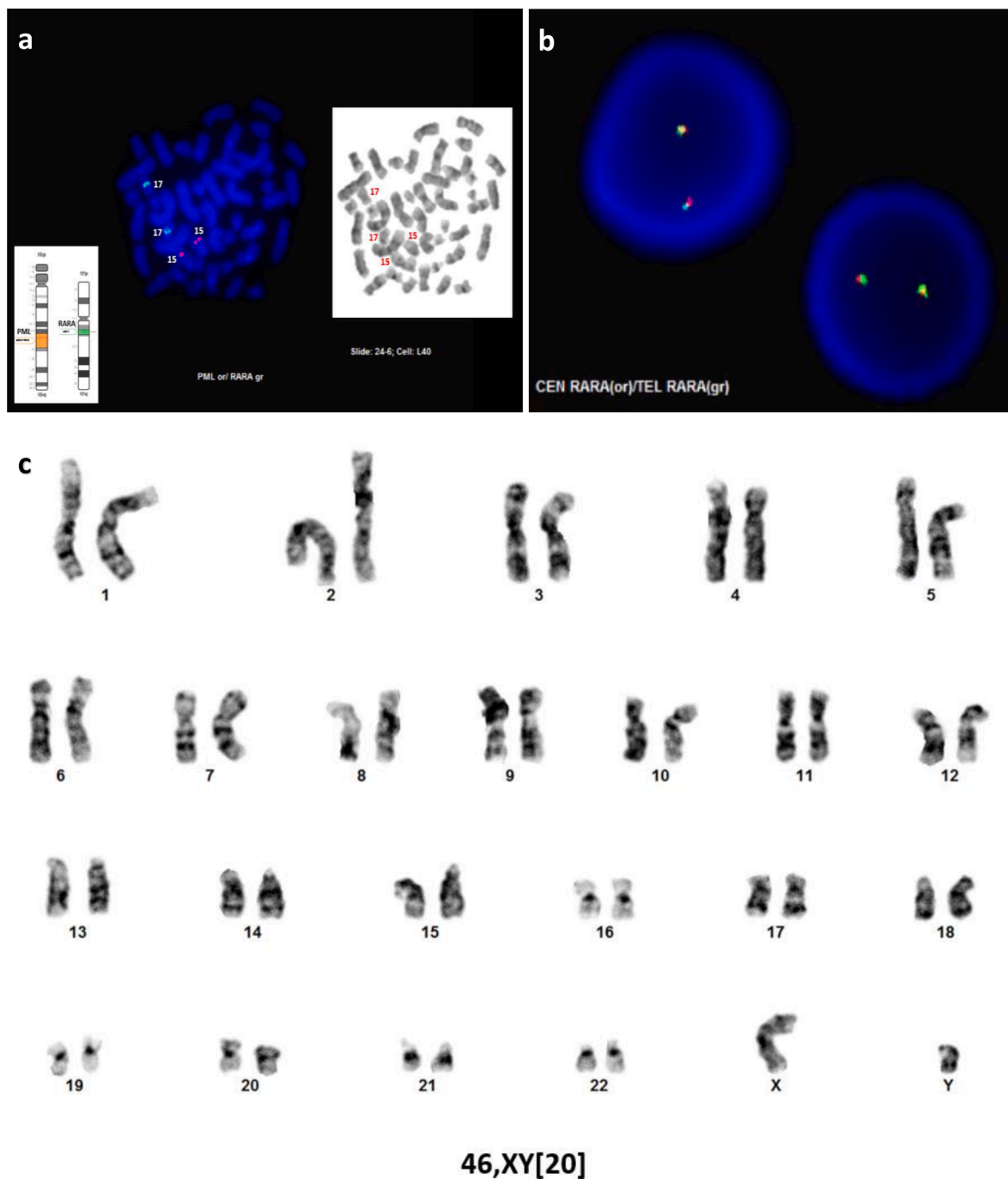


Fig. 2. Cytogenetic findings of APL with cryptic *PML::RARA* fusion.

proposed ancillary techniques utilized for the detection of the *PML::RARA* fusion. In brief, the most popular is RT-PCR, which is important for isoform identification as well as evaluation for measurable residual disease at later points. However, other techniques have been employed such as Sanger sequencing [4], whole-genome sequencing (WGS) [5], and mate-pair sequencing (MPS) [6]. Unfortunately, each of these methodologies is not without shortcomings. The most commonly employed method for detecting the *PML::RARA* fusion is RT-PCR but RT-PCR primers targeting the *PML::RARA* fusion will fail to detect variant *RARA* fusions with other genes, such as *ZBTB16*, *NUMA1*, *NPM1*, and *STAT5B*. As such, employing only RT-PCR without other methodologies may lead to false negative diagnoses, thus delaying or even failing to initiate the appropriate therapy. In the context of Sanger

sequencing, WGS, and MPS, these methodologies are incredibly useful for identifying cryptic *PML::RARA* fusions, but these methods have a long turnaround time. In particular to WGS and MPS, these assays are expensive and not available in all molecular diagnostic laboratories.

In the era of targeted NGS panels and given the rarity of this phenomenon, a knowledge gap currently exists in terms of single nucleotide variants (SNV) and insertions/deletions (indels) that have been identified in conventional APL but are poorly characterized in APL with cryptic *PML::RARA* fusions. Notably, the present case demonstrated a *FLT3* p.D835Y variant within the tyrosine kinase domain while other SNV or small indels were not detected. Upon review of our institution's cohort of 12 conventional APL cases from 2017 to 2021, five cases had a targeted NGS panel performed which revealed *FLT3* p.D835E, *WT1* p.

A382fs, *SETD2* p.T168I, and *NRAS* p.G12S variants in three patients, thus potentially demonstrating the genetic heterogeneity of APL regardless of the cryptic nature of the *PML::RARA* fusion (unpublished data). Additionally, within our cohort, *FLT3* analysis by PCR demonstrated that of six cases, five had a *FLT3* internal tandem duplication (ITD). The finding of *FLT3* ITD has been reported previously in two APL cases in which the *PML::RARA* was only identified by RT-PCR [7, 8], but a *FLT3* TKD variant has not been reported to date.

As there is potential genetic heterogeneity amongst the cryptic *PML::RARA* APL group, Kim et al. evaluated 13 previously documented cases of cytogenetically cryptic *PML::RARA* in APL with a few key genetic features identified, such as the predominance of the short (bcr3) isoform and additional chromosomal aberrations [9]. Rashidi and Fisher revealed a similar predominance of the short isoform as well as chromosomal aberrations present in 52% of reported cases [10]. Although there appears to be a mild trend toward a specific isoform as well as additional chromosomal aberrations, more data needs to be collected on this unique entity and it will be of great interest to analyze the genomic profile of future cases to perhaps elucidate any additional molecular features.

Herein, we have provided a case of APL with a cryptic *PML::RARA* fusion as identified by RT-PCR with review of relevant elements of the English literature, as well as providing additional NGS data. As such, a unifying clinical or genomic feature for APL with a cryptic *PML::RARA* fusion remains elusive. Of practical interest, the present case underscores the necessity for employing multiple testing methodologies for identifying the *PML::RARA* fusion, particularly when clinical and pathologic suspicion is high. Finally, as molecular techniques have evolved, new technologies such as optical genome mapping may be of interest and applicability for identifying potentially cryptic, as well as variant, translocations.

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Ethics approval

The Beth Israel Deaconess Medical Center Institutional Review Board has approved this study with a protocol no. 2022P000026 and informed consent was obtained from the patient.

Author statement

K.K. analyzed data, reviewed the literature, wrote and revised the manuscript; C.B. analyzed the cytogenetic data, provided images, and reviewed the manuscript; A.D. provided clinical data and reviewed the manuscript; P.M. reviewed the case, analyzed data, wrote and revised the manuscript. K.K., C.B., A.D., and P.M. approved the manuscript.

Declaration of Competing Interests

The authors have no competing interests.

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