Case of the Quarter - Aug 2013

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Diagnosis: Acute Myeloid Leukemia, NOS,
with MYC Amplification and Promyelocytic Differentiation

Introduction:

Double minutes (dmin) and homogeneously staining regions (hsr) are the cytogenetic hallmarks of genomic amplification in cancer, more common in solid tumors than in hematologic malignancies. Double minute chromosomes are seen primarily in acute myeloid leukemia, with amplification of the MYC oncogene or, less frequently, the MLL transcription factor (Rayeroux and Campbell 2009). Both normal and abnormal karyotypes were reported together with dmin, and rarely with hsr. It is associated with a poor prognosis, rapid disease progression and short survival, however only in case reports and small series (Thomas et al, 2004, Receveur et al 2004).

Here we present a case of AML with morphologic and immunophenotypic features resembling acute promyelocytic leukemia (APL) that has amplification of MYC present on dmin but no translocation, t(15;17)(q22;q12) or fusion of PML-RARA.

Clinical History:
The patient is a 67 year old man who presented with a several month history of shortness of breath, increased bruising, and acute swelling of his legs and toes. He reported frequent bruises on his arms and legs, but denied gingival bleeding. Ultrasound evaluation of the lower extremities for deep vein thrombosis was negative. His CBC with manual differential counts revealed mild anemia, thrombocytopenia and circulating immature myeloid forms with large number of promyelocytes (Hgb 12.2 g/dl, Plt 80 k/µl, WBC 11 k/µl with 24% promyelocytes, 26% myelocytes, 3% metamyelocytes, 24% neutrophils, 18% lymphocytes, 3% monocytes) and coagulation studies were within normal limits (PT 14.0, INR 1.1, PTT 29.1, Fibrinogen 300 µg/L).

After an initial bone marrow examination, the patient was admitted with the presumptive diagnosis of APL and initiated treatment with all-trans retinoid acid (ATRA) on admission. After the results of the full acute leukemia workup became available, his chemotherapeutic regimen was changed from ATRA to 7+3 induction chemotherapy with cytarabine and daunorubicin. A subsequent bone marrow biopsy revealed persistent disease and re-induction was initiated. His clinical course has since been complicated by neutropenic fevers and small bowel obstruction.

**Acute Leukemia Workup:**

**Pathology of bone marrow aspirate and biopsy**

Examination of the bone marrow aspirate smear revealed immature myeloid forms, many with abundant eosinophilic granules (Figure 1A). The bone marrow differential showed 83% blasts and abnormal promyelocytes, 10% maturing myeloids, 4% erythroids and 3% lymphoid cells. Auer rods are not seen and unlike classic APL, the abnormal promyelocytes do not have bilobed nuclear contour. They also lack prominent Golgi and have prominent nuclei and aggregates of eosinophilic granules. Histochemistry stain showed heavy myeloperoxidase staining and negative for non-specific esterase (Figure 1B and data not shown). Bone marrow biopsy revealed hypercellular marrow with background fibrosis and aggregates of immature myeloid cells with round nuclei and prominent nucleoli, and abundant cytoplasm - often with numerous granules (Figure 2).

**Flow Cytometric analysis**

6-color analysis with CD45-gating revealed that the majority of the immature cells with the following phenotype: CD45(dim), CD34-, CD117-, HLA-DR-, CD33+, CD15(bright), CD64(dim), myeloperoxidase+, CD13-, and CD11B-.

**Cytogenetics and FISH studies**

On karyotype analysis, none of the 20 metaphases analyzed had a t(15;17) or other known AML-associated rearrangements. 3 of the 20 had multiple double-minute chromosomes (Figure
3) and other 13 abnormal metaphases contained a paracentric inversion of the short arm of chromosome 1, as the sole aberration (data not shown).

FISH evaluation for MYC amplification was performed on nuclei with the MYC Dual Color, Break Apart Probe at 8q24 and MYC amplification was observed in 7/100 nuclei (Figure 4). FISH evaluation for PML-RARA rearrangement with the PML/RARA Dual Color, Dual Fusion Probe for PML at 15q24 and RARA at 17q21.1 showed no rearrangement in 100 nuclei.

**Molecular Diagnostics**

RT-PCR for BCR-ABL or PML-RAR fusion transcripts were both negative. DNA Sequence analysis FLT3 ITD, FLT3 D835, NPM1 exon 12 insertions and CEBPA mutations are all negative.

A repeat bone marrow biopsy after induction revealed the persistent presence of immature cells, with an increased number of cells containing granules compared to the prior evaluation (data not shown).

**Discussion:**

Since patients with APL frequently present with potentially life-threatening coagulopathies and require rapid diagnosis and treatment with all-trans retinoic acid (ATRA) (1, 2). Patients with leukemic cells with morphologic features similar to APL are often given ATRA while waiting for confirmation of the diagnosis of APL. A translocation of chromosomes 15 and 17 is identified in the vast majority of cases that present with morphologic and immunophenotypic features of APL. However, occasional cases present without this classic translocation could have an alternative form of the PML-RARA fusion or very rarely, the presence of PML-RARA fusion can not be demonstrated but patients still responded to ATRA clinically. Thus, the use of multiple methods including both cytogenetic and molecular techniques is critical for determining whether a cryptic PML-RARA fusion is present in order to guide appropriate treatment. In this case, both cytogenetic methods and molecular methods did not identify the PML-RARA fusion but revealed the amplification of MYC that likely plays a key role in oncogenesis.

This case represents an unusual AML with MYC amplification. MYC is a transcription activator with many target genes involved in key processes controlling cellular proliferation and differentiation. Increased level of MYC protein is observed in multiple cancer types and can be the result of chromosomal rearrangement, gene amplification or transcriptional activation. In AML or myelodysplastic syndromes (MDS), structural changes of the MYC locus are uncommon. Increased level of MYC expression is generally attributed to transcriptional up-regulation through activation of other oncogenic pathways. Review of the literature shows several reports of AML with alteration of the MYC locus in the forms of amplification, either on double-minutes or homogenously staining region (Rayeroux KC, Campbell LJ, 2009, Thomas et al 2004, Christacos et al 2005, Frater et al 2006, Villa et al 2008, Lee et al 2009, Bruyer et al 2010, Angelova et al 2011, Yamamoto et al 2013). On the other hand, trisomy 8 is common in
myeloid neoplasms and one copy increase of MYC is present in many AML and MDS secondary to trisomy 8.

In most reported cases with MYC amplification, the bone marrow morphology showed AML with maturation. In this case, the majority of the leukemic cells were arrested at the promyelocyte stage, but they lacked the characteristic bilobed nuclei and prominent Golgi apparatus typically seen in cases of APL. Furthermore, although several cells contained eosinophilic structures suggestive of Auer rods, no classic Auer rods or Faggot cells were seen. So far, there are a few sparse case reports of patients with APL-like morphology and MYC amplification, (old cases reviewed in Frate et al 2006, Kamath et al 2008, Bruyer et al 2010, The absence of PML-RARA rearrangement was demonstrare by karyotype, FISH analysis and/or RT-PCR in all except in two old reports.

There are at least two other known case of a patient with APL-like morphology and the presence of C-MYC amplification but the absence of a PML-RARA rearrangement (4, 16). It is unclear what characteristics of this cellular genotype results in the morphologic and immunophenotypic appearance of promyelocytes.

Figures:

Figure 1A. Wright-Giemsa stain of the bone marrow aspirate at 100x magnification. The majority of marrow cellularity is composed of blasts and abnormal promyelocytes with nucleoli and abundant azurophilic granules but rare Auer rods.
Figure 1B. Myeloperoxidase stain of the bone marrow aspirate at 100x magnification. Most of the leukemic cells are intensely positive for myeloperoxidase.
Figure B. Hematoxylin and Eosin stain of the bone marrow biopsy at 100x magnification. Leukemic cells have round to oval nuclei, distinct nucleoli and moderate amount of cytoplasm with eosinophilic granules.
Figure 3: Karyotype of cells with multiple double minutes and no t(15;17) on initial presentation.
Figure 4: Fluorescent in-situ hybridization with MYC Dual Color, Break Apart Probe at 8q24. The presence of multiple aggregates of green and red signals dispersed in the interface nuclei, consistent with amplification present on double-minutes.
References:


Frater JL, Hoover RG, Bernreuter K, Batanian JR. Deletion of MYC and presence of double minutes with MYC amplification in a morphologic acute promyelocytic leukemia-like case lacking RARA rearrangement: could early exclusion of double-minute chromosomes be a prognostic factor? Cancer Genet Cytogenet. 2006 Apr 15;166(2):139-45


Mi JQ, Li JM, Shen ZX, Chen SJ, Chen Z. How to manage acute promyelocytic leukemia. Leukemia. 2012 Aug;26(8):1743-51


