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Mixed Phenotype Acute Leukemia with Low Hypodiploidy in a Pediatric Patient

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Abstract

We describe the case of a 16 year-old female with mixed phenotype acute leukemia B/myeloid, NOS (formerly biphenotypic leukemia) with masked hypodiploidy and somatic *TP53* and *CDKN2A/B* deletions. She achieved morphologic remission with lymphoid-directed multi-agent chemotherapy, but experienced an early medullary relapse 11 months from initial diagnosis. Her case details the unusual finding of hypodiploidy in a patient with ambiguous lineage leukemia and highlights the complexity of therapy selection for these high-risk patients.

Keywords

Biphenotypic leukemia; hypodiploidy; mixed phenotype acute leukemia

1. INTRODUCTION

Acute leukemia of ambiguous lineage (ALAL) is a rare diagnosis associated with complex clinical, cytogenetic, immunophenotypic, and/or molecular genetic features and with

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CONFLICTS OF INTEREST

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adverse clinical outcomes [1]. According to the World Health Organization 2008 classification, ALAL includes several rare leukemia subtypes, such as acute undifferentiated leukemia (leukemia without lymphoid or myeloid markers) and mixed phenotype acute leukemia (MPAL; leukemias with co-expression of dual lineage antigens or with two distinct clonal monolineage blast populations) [2]. Representing approximately 2% of all acute leukemias, MPAL has been described in adults and children with a male predominance [1,3,4]. Patients with MPAL have poor responses to lymphoid- and myeloid-directed chemotherapy and experience high rates of relapse [3,5,6]. Recent analyses estimate that 10–30% of patients with MPAL exhibit complex clonal chromosomal abnormalities, including trisomies of chromosomes 4 and 8 or occasional *ETV6-RUNX1*, *MLL*, and *BCR-ABL* rearrangements [1,4,5]. The prognostic significance of such cytogenetic abnormalities remains unknown in MPAL given its rarity and the lack of unifying genetic alterations.

Hypodiploid chromosome number is typically associated with children with B acute lymphoblastic leukemia (B-ALL) with event-free survival (EFS) estimates ranging from 20–40% [7–9]. Outcomes appear to be associated with degree of hypodiploidy, as worse event-free survival occurs with decreasing number of chromosomes in the leukemias [10]. Hypodiploid leukemias may undergo chromosomal doubling such that the total chromosome number appears falsely hyperdiploid, leading to misclassification as high hyperdiploid ALL with favorable prognosis. Recent data demonstrate that low hypodiploid clones may be associated with *TP53* deletions (somatic or germline) and high rates of early medullary relapse [7, 10, 11]. Despite recent improvements in understanding hypodiploid B-ALL biology, co-occurrence of hypodiploidy and ALAL remains a novel finding to our knowledge. We describe the first known case of a pediatric patient with an MPAL with masked duplicated low hypodiploidy and a somatic *TP53* deletion.

2. RESULTS

A previously healthy 16 year-old female of Indian ethnicity presented initially to an Indian hospital with a 3 month history of bone pain, fever, weight loss, menorrhagia, and rash. She had an initial white count of 4.0 × 109/L, and no central nervous system involvement with leukemia. Flow cytometric analysis of her bone marrow demonstrated blasts positive for CD45, CD34, TdT, HLA-DR, CD13, CD33, dim for CD79a, and negative for CD10, CD19, CD20, and cMPO. CD22 was not tested, and all T-cell markers were negative. Bone marrow aspirate immunostaining was positive for TdT, CD24, and Pax-5 and negative for CD10, CD20, and MPO. She was thus diagnosed with pro-B ALL with aberrant CD13 and 33 expression. Cytogenetics were notable for a 46,XX karyotype with no monosomies noted. Reverse transcriptase polymerase chain reaction (RT-PCR) testing was negative for *BCR-ABL1* and *MLL* rearrangements and for 26 other common acute myeloid leukemia (AML)- and ALL-associated translocations. She underwent a 4-drug induction with dexamethasone, vincristine, daunorubicin, and L-asparaginase and triple intrathecal chemotherapy. Her induction day 15 bone marrow demonstrated hypocellular morphology with rare blasts.

The patient transferred to a Singaporean institution where review of initial leukemia pathology was interpreted as more consistent with a diagnosis of MPAL given a subset of MPO+ blasts. Fluorescence *in situ* hybridization assays performed there on initial diagnostic

marrow slides revealed additional *RUNX1* and *RUNX1T1* signals, but no *RUNX1-RUNX1T1* fusion resulting from a t(8;21) (Figure **1A**). Repeat bone marrow completed 6 weeks from diagnosis showed no evidence of disease by morphologic and flow cytometric testing. She subsequently received consolidation therapy with cyclophosphamide and cytarabine given her favorable response to lymphoid-directed induction chemotherapy. Due to the conflicting diagnoses at these two institutions, however, the patient sought a second opinion at the Children's Hospital of Philadelphia for further treatment recommendations. Based solely upon the available written reports from both institutions, our hematopathologists deemed her diagnosis more consistent with AML not otherwise specified (NOS). Repeat bone marrows performed here at that time were negative for AML and B-ALL by morphology and flow cytometric minimal residual disease (MRD) testing. Given our diagnosis of AML NOS, we recommended myeloid-directed therapy with several cycles of cytarabine, anthracyline, and etoposideShe returned to her treating Singaporean institution and received 3 cycles of therapy with a first cycle of cytarabine, daunomycin, and etoposide, a second cycle of attenuated high-dose cytarabine with L-asparaginase, and a third cycle of high-dose cytarabine.

Four months after chemotherapy completion, the patient re-presented to our institution due to worsening thrombocytopenia. Bone marrow aspirate, biopsy, and extensive leukemia genetic testing (cytogenetics, whole genome single nucleotide polymorphism (SNP) microarray, targeted hematologic malignancies DNA sequencing mutation analysis) were performed given the initial complexity of her leukemia diagnosis and potential leukemic evolution upon relapse. Bone marrow morphology from the relapse specimen contained 83.5% large, vacuolated leukemic blasts and no visible Auer rods [12]. Flow cytometric immunophenotyping confirmed a B/myeloid MPAL with blasts brightly positive for CD45, CD34, and CD19, variably positive for CD9, CD38, CD24, and CD15, and subset positive for sCD22, cCD79a, TdT, and MPO. CD10, CD2, CD3, CD5, CD7, and CD8 staining was negative. Cytogenetic analyses demonstrated 65 chromosomes in the leukemic cells. SNP array analysis demonstrated loss of heterozygosity and two copies of chromosomes 3, 4, 5, 7, 9, 13, 15, 16, 17, 20, and X, trisomies of chromosomes 1, 2, 10, 18, and 19, and tetrasomy of chromosomes 6, 8, 11, 12, 14, 21, and 22 (Figures **1** and **2**). These results are consistent with doubling of a low hypodiploid clone with 35 chromosomes, with subsequent loss of one copy of chromosomes 1, 2, 10, 18 and 19.

Mutation analyses for AML-associated internal tandem duplication of the fms-like tyrosine kinase receptor 3 (*FLT3*-ITD), *CEBPA* point mutations, and *NPM1* point mutations were negative. Results from the targeted leukemia mutation analyses revealed a nonsense mutation in *TP53*. Germline *TP53* mutation testing was recommended to evaluate for possible Li-Fraumeni syndrome, but was not performed to our knowledge. Following our evaluation, the patient transferred care to another American institution, where she was treated with two cycles of salvage therapy, including one with dexamethasone, thiotepa, topotecan, and vinorelbine. The patient is currently in an MRD negative remission with plans to undergo matched unrelated donor bone marrow transplant.

3. DISCUSSION

To date, only one other case of MPAL with hypodiploidy has been reported. Tallents *et al.* described a 37 year-old man who initially presented with B-ALL, relapsed 4 months later on therapy, and was found to have a hybrid MPAL with masked duplicated low hypodiploidy (70 chromosomes) [13]. To our knowledge, our patient is the first case of childhood ALAL with hypodiploidy reported in the literature.

A variety of chromosomal abnormalities have been associated with MPAL; however, true hypodiploidy has not been reported [1,4,5]. Chromosomal abnormalities associated with MPAL include monosomies of chromosomes 5 or 7, trisomies of chromosomes 4, 19 and 21, polysomies of chromosome 8, and hyperdiploidy. Deletions associated with MPAL include those in 1p32, 5q, 6q, 7q, and 12p. Other fusion genes found in patients with ALAL include *BCR-ABL1*, *ETV6-RUNX1*, *NUP98-IQCG*, or *MNX1-ETV6*, and *MLL* rearrangements [1, 3, 4]. This patient had trisomy 19 and tetrasomy 8, which have been reported in children and are also associated with AML. While polysomy 8 has been reported in a case of myeloid/B or T lymphoid MPAL and may have a more favorable prognosis, trisomy 19 is associated with myeloid/T lymphoid MPAL and is of uncertain significance [1,3]. Despite these similarities, the hypodiploidy observed in this patient remains a novel finding in a patient with MPAL.

This patient's leukemia hypodiploidy also occurred in the context of a somatic *TP53* mutation, a recently characterized genetic finding in low hypodiploid ALL [10]. Muhlbacher *et al.* also described 29 cases of low hypodiploid ALL that were characterized by frequent duplication of the leukemic chromosomes, losses of chromosomes 3, 7, 13, 15, 16, and 17, *TP53* and *CDKN2A/B* deletions, as well as poor event-free and overall survival [11]. In this patient, the hypodiploid clone doubling, somatic mutations, and poor response to chemotherapy are consistent with these previous descriptions of hypodiploid ALL with *TP53* mutation.

Hypodiploidy is associated with poor prognosis in both childhood ALL and adult AML [8, 14]. While hypodiploid ALL comprises approximately 2% of childhood ALL, a recent meta-analysis reported that 22% of *de novo* AML cases in adults are hypodiploid [9, 10, 15]. This patient's early medullary relapse suggests that her MPAL demonstrates a similar poor prognosis to those of both hypodiploid ALL and AML.

Although the patient's *de novo* leukemia cytogenetics were reported as normal at the original treating institution, subsequent testing of her diagnostic material reveal some evidence of chromosomal number abnormality by FISH. It remains unknown whether her leukemia evolved at time of relapse with a predominant hypodiploid clone and if her original cytogenetics data represented normal cells and not leukemia. Interestingly, her leukemia flow cytometric immunophenotyping and immunohistochemical data were identical from specimens at diagnosis and relapse, suggesting that her MPAL was likely the same disease with minimal genetic evolution.

In summary, the combination of MPAL with masked doubling of a hypodiploid clone and a *TP53* mutation demonstrates an unusual genetic finding in a childhood leukemia patient that is likely associated with high risk of relapse and poor outcome.

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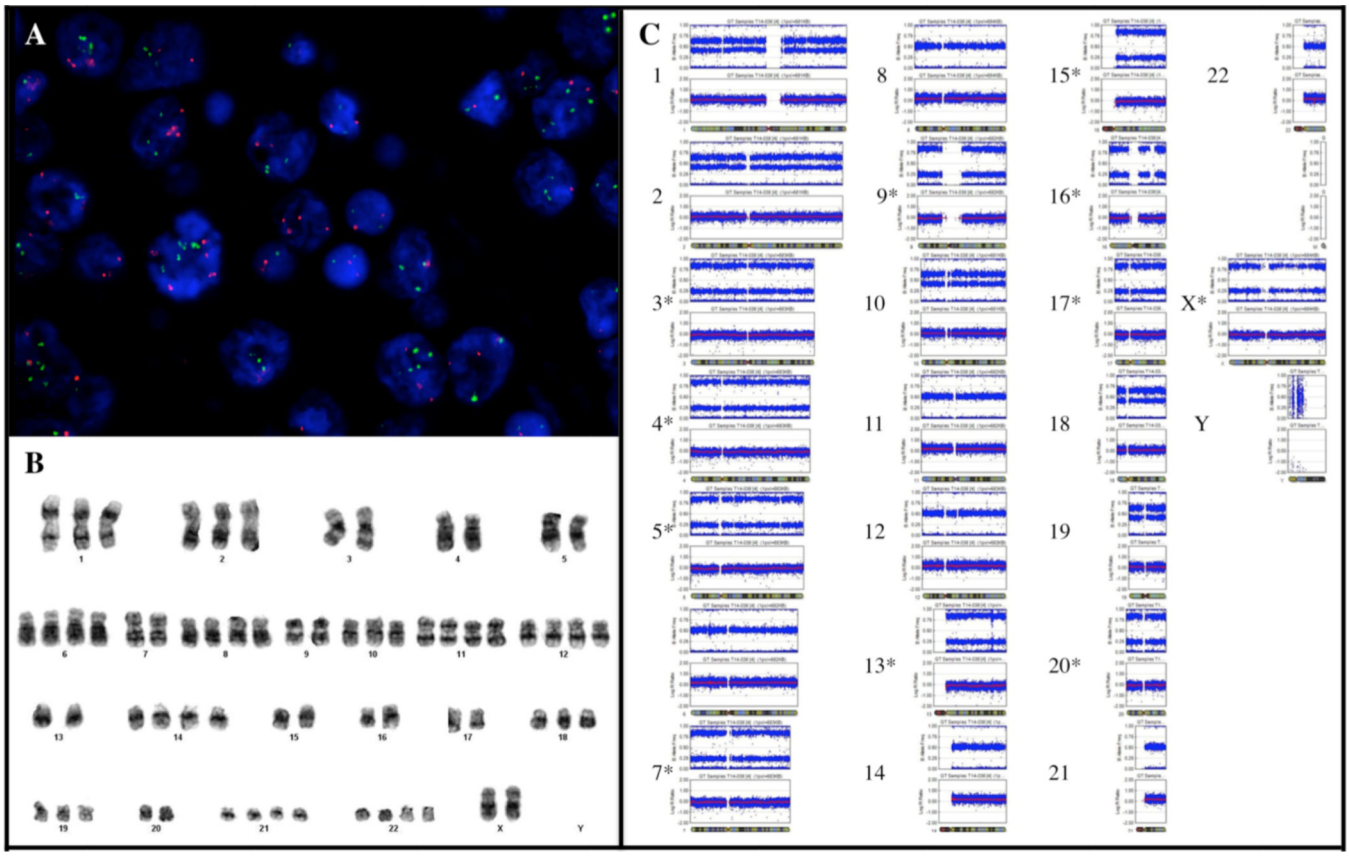

Figure 1.

FISH of initial leukemia specimen and genetic analyses of relapsed leukemia specimen. **A**. Fluorescence *in situ* hybridization assays for *RUNX1* (red) and *RUNX1T1* (green) on initial diagnostic marrow (Credit: Dr. Alvin Lim, the Cytogenetic Laboratory, Singapore General Hospital). **B**. Representative G-banded karyotype of the leukemic clone from bone marrow aspirate at relapse. **C**. SNP array of relapsed leukemia specimen. Loss of heterozygosity on chromosomes 3, 4, 5, 7, 9, 13, 15, 16, 17, 20, and X (indicated with *) was observed.

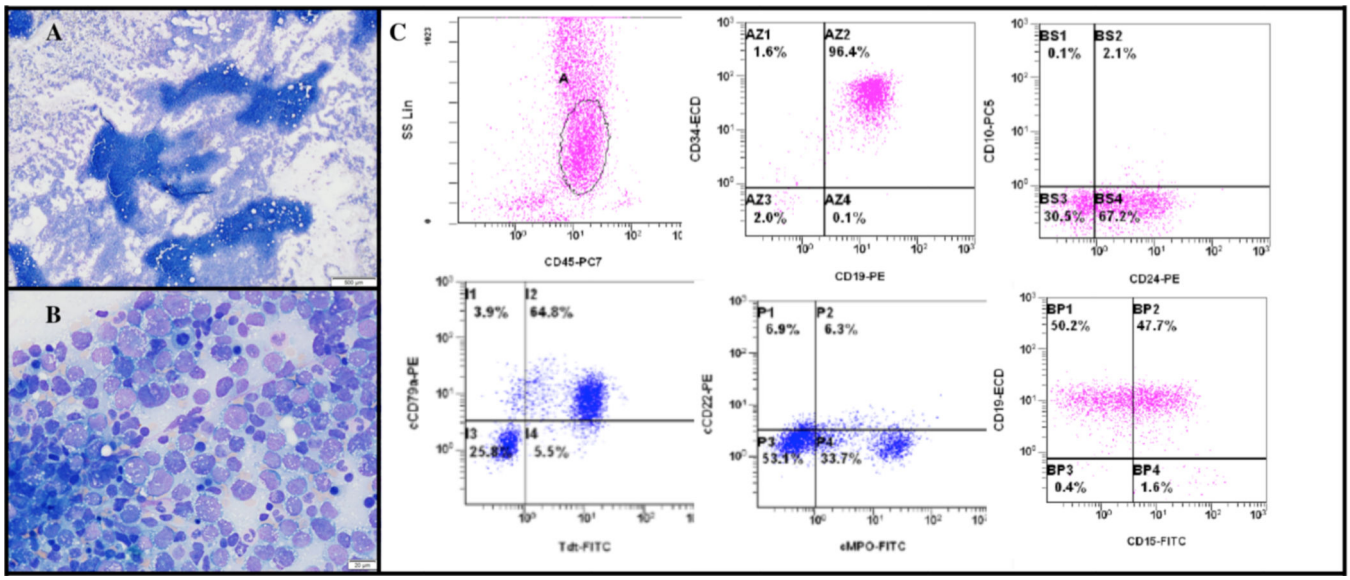

Figure 2.

Histopathology and flow cytometry of relapsed leukemia specimen. A bone marrow aspirate showing a densely packed marrow (**A**) as well as large, atypical, vacuolated blasts (**B**). Flow cytometry (**C**) of relapsed leukemia was positive for B cell markers including CD19, CD24, CD22, and cCD79a, as well as positive for myeloid markers MPO and CD15. CD10 is negative. (**A**) Wright-Giemsa, ×40; (**B**) Wright-Giemsa × 600.