Case Study

Myelodysplastic syndrome following precursor B-cell acute lymphoblastic leukemia with low hypodiploid / near triploid karyotype and concomitant *TP53* mutation: A case report and review of the literature

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Abstract: Low hypodiploid / near triploid acute lymphoblastic leukemia (LH-ALL) is infrequent in children with precursor B-cell acute lymphoblastic leukemia, and development of a secondary malignancy, particularly myelodysplastic syndrome (MDS), is additionally uncommon. Here we report a case of a 12-year-old girl who initially presented with a precursor B-cell acute lymphoblastic leukemia characterized by a karyotype with a chromosome count of 34 accompanied by a near triploid clone with 63 chromosomes. These findings prompted a *TP53* mutation study, which found a deleterious mutation (524G→A) resulting in an amino acid substitution of R175H (Arg175→His). Two years after successful treatment of the LH-ALL a bone marrow examination demonstrated dyserythropoiesis, dysmegakaryopoiesis, increased hematogones, and a 7q deletion consistent with MDS. The development of MDS following treatment of LH-ALL is exceptionally rare, with only one other case found in our search of available literature.

Keywords: Myelodysplasia, acute lymphoblastic leukemia, low hypodiploid / near triploid karyotype, *TP53* mutation.

Introduction

Low hypodiploid / near triploid acute lymphoblastic lymphoma (LH-ALL) is rare in children with precursor B-cell acute lymphoblastic lymphoma, and is associated with *TP53* mutations, Li-Fraumeni syndrome, and poor prognosis. Development of a secondary malignancy, particularly myelodysplastic syndrome (MDS), is additionally uncommon and studies concerning its pathogenesis and clinical outcomes are scarce. Here we report a case of LH-ALL in a 12-year-old girl with a *TP53* mutation followed 2 years later by MDS.

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Case Report

Case History

The patient is a 12-year-old girl who presented with several weeks of weight loss, along with ecchymosis, petechiae and episodes of epistaxis to our Pediatric

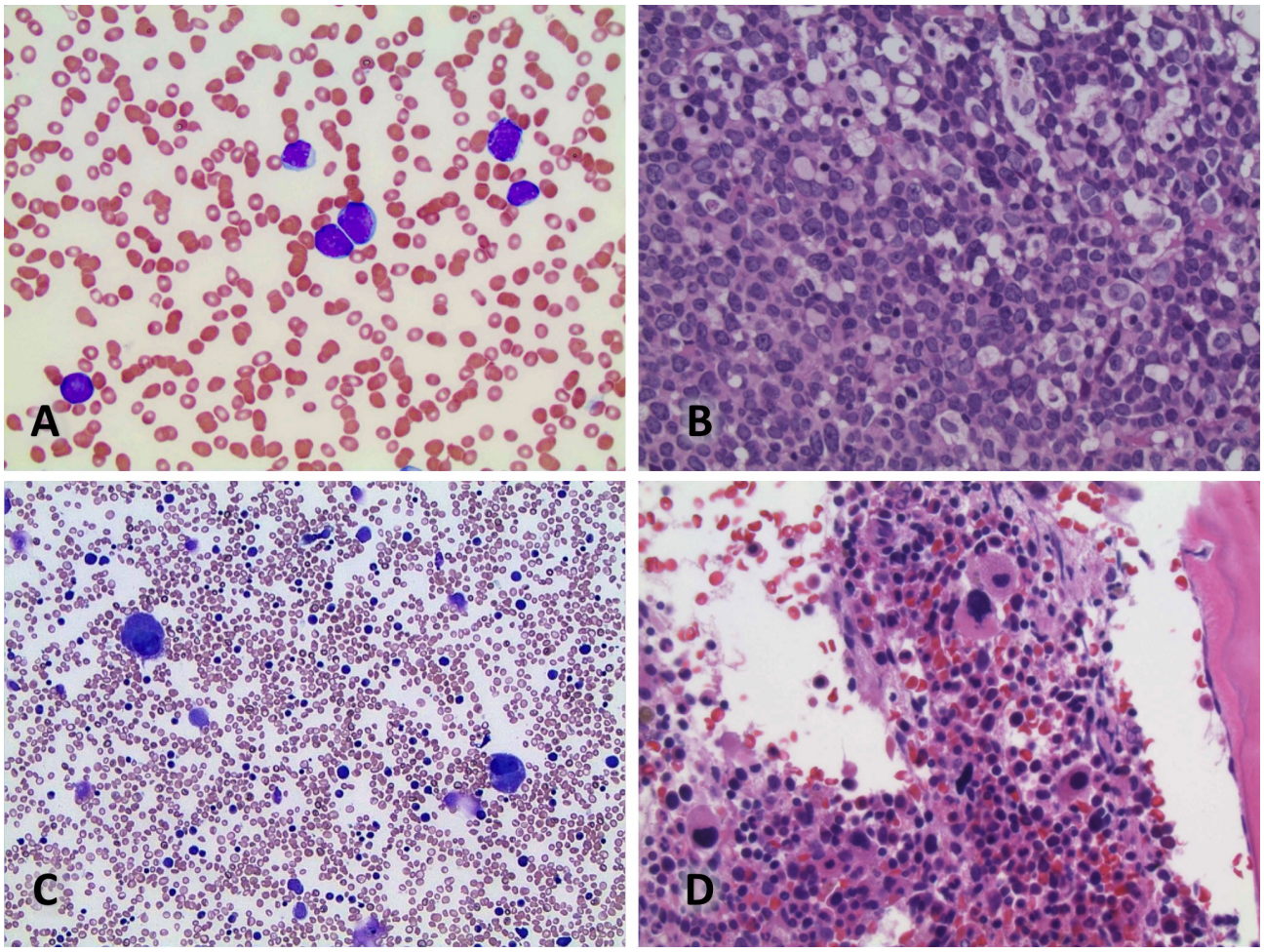

Figure 1: (A) Initial peripheral blood smear with many circulating blasts (Wright-Giemsa stain, original magnification x400). (B) Precursor B-cell acute lymphoblastic leukemia: initial marrow biopsy (Hematoxylin and Eosin stain, original magnification x400). (C) Dysmegakaryopoiesis in the bone marrow aspirate and (D) biopsy two years after induction chemotherapy ([C] Wright- Giemsa stain, original magnification x200; [D] Hematoxylin and Eosin stain, original magnification x400).

Emergency Department. A complete blood count demonstrated severe microcytic anemia (hemoglobin 8.4 g/dL) with thrombocytopenia (platelets 10 x 10^9/L) and leukocytosis (white cell count 21.24 x 10^9/L) with 60% circulating blasts [Figure 1A]. Lactate dehydrogenase was elevated at 1534 U/L, and chest radiographs were essentially normal. A bone marrow biopsy and aspiration demonstrated blasts in the marrow with morphology similar to those seen in peripheral blood. With supporting ancillary tests (see Pathologic and Genetic Findings) a diagnosis of low hypodiploid / near triploid precursor B-cell acute lymphoblastic leukemia (LH-ALL) was made, and she was started on COG protocol AALL1131. Repeat bone marrow examinations were consistent with remission marrow after induction. Complications of her hospital course included dehydration, a port-a-cath infection, hydrocephalus due to methotrexate toxicity, and cholestatic jaundice.

Two years later, she again began to bruise easily and had petechiae on her legs. A complete blood count at that time showed hemoglobin of 10.9 g/dL,

platelets 52 x 10^9/L, and white cell count 3.57 x 10^9/L. Lactate dehydrogenase was within normal range (170 U/L). Bone marrow evaluation at this time was consistent with low grade myelodysplasia, i.e., refractory cytopenia with multilineage dysplasia.

As of this writing, the patient is being evaluated for a bone marrow transplant, but has had elevated liver enzymes due to iron overload per liver biopsy results.

Pathologic and Genetic Findings

Although no aspirate smear was available for evaluation, the initial trephine core biopsy sections as well as the touch imprints demonstrated a hypercellular marrow with nearly total replacement by an atypical blast population (95%). The blasts were monomorphic, medium-sized cells with scant cytoplasm, high nuclear / cytoplasm ratio, dispersed chromatin and prominent nucleoli [Figure 1B]. Megakaryocytes, granulocytes, and erythroid precursors were not distinguishable in the sheets of atypical cells. By flow cytometry, the abnormal blast population expressed CD10, CD19, CD20, cytoplasmic CD22, surface CD22 dim, CD34 partial, CD38, CD45 partial, CD58, CD79a, HLA-DR, and TdT, but not expressing T cell or myeloid antigens. The morphologic and immunophenotypic findings were most consistent with precursor B-cell acute lymphoblastic leukemia (pre-B ALL). Chromosomal analysis of 20 metaphases from the peripheral blood demonstrated 12 metaphases which were hypodiploid with a chromosome count of 34, and 6 metaphases that were "pseudo-hyperdiploid", due to a "doubling of the hypodiploid clone", and had a chromosome count of 63. FISH studies confirmed that 65% of the nuclei evaluated had monosomy 4, 9, 12, 17 and 22 consistent with the hypodiploid clone identified in the chromosome studies. In addition, approximately 13% of nuclei had tetrasomy 1, 10, 14, 19, and 21 and trisomy 11, which is consistent with the pseudo-hyperdiploid clone also identified in the chromosome studies. Five months later, while the patient was in remission, a *TP53* full gene analysis on peripheral blood demonstrated a deleterious mutation with DNA change of c.524G>A and an amino acid change of p.R175H (Arg175His), consistent with the diagnosis of Li-Fraumeni syndrome.

Histological examination of the bone marrow two years after finishing induction therapy showed a mildly hypocellular marrow for age (~80% overall cellularity) with a moderate megakaryocytic hyperplasia with dysmegakaryopoiesis and dyserythropoiesis and a mild increase in hematogones [Figure 1C and 1D]. By flow cytometry, the bone marrow aspirate showed a mild increase in hematogones (~14% of total events), but no evidence of increased myeloblasts (1% of total events), aberrant antigen expression, or acute lymphoblastic leukemia. Chromosomal analysis of 20 metaphases on the bone marrow now demonstrated 14 metaphases with several structural abnormalities, including an unbalanced t(5;17) translocations resulting in deletion 5q and 17p, and duplication 11q. FISH studies demonstrated a *TP53* gene deletion and a 5q deletion in approximately 77% of nuclei. Additionally, a 7q deletion was observed in 22.5% of nuclei. These results were concerning for an emerging, possible therapy-related, myeloid clone involving the marrow, such as MDS.

Discussion

Acute lymphoblastic leukemia (ALL) is one of the most common malignancies in patients under 20 years of age [1], the majority of which (85-90%) are of the B-cell lineage [2]. Despite having high 5-year event-free survival rates, ALL is the leading cause of cancer-related death in children and young adults (aged 21-39) [3]. Adults with ALL have a significantly worse prognosis than children with ALL, which is most likely due to unfavorable genetic alterations [3]. Up to 80% of ALLs express aberrant karyotypes, which can be divided into two major subgroups: (1) ALL with chromosomal rearrange-

ments leading to leukemia-specific fusion genes, and (2) gains and losses of whole chromosomes, known as *aneuploidy* [4]. The second subgroup (aneuploidy) is further divided into "ploidy groups," with nomenclature based on the number of chromosomes relative to a normal karyotype with 46 chromosomes [4]. For example, high hyperdiploidy ALL (H-ALL) has more than 50 and less than 60 chromosomes and most commonly results from gains in chromosomes 4, 6, 10, 14, 17, 21 and X [3, 4]. H-ALL constitutes up to 30% of childhood B-cell precursor ALL and is associated with an excellent prognosis [3]. Alternatively, low hypodiploid karyotypes can have between 25 and 40 chromosomes, are only found in about 3% of childhood B-cell precursor ALL, and are associated with a poor prognosis [3, 4]. A simple count of chromosomes, however, may be misleading as nearly half of patients with low hypodiploid karyotypes in one study had near triploid clones that had between 56 and 78 chromosomes [4]. As was found in our patient, near triploid clones show nearly the same pattern of chromosomal losses as the low hypodiploid clone they are found with, and the increased number of chromosomes may be attributed to doubling of the low hypodiploid clone [4]. The chromosomes typically lost in LH-ALL are 3, 4, 7, 9, 12, 13, 15, 16, and 17, while chromosomes 1, 6, 10, 19, 21, 22 and the sex chromosomes are commonly retained [4]. Our case showed a very similar pattern of chromosomal loss and retention, and the near triploid clone showed doubling or tripling of the retained chromosomes.

TP53 mutations have been found in more than 90% of LH-ALL at initial diagnosis [4, 7], which only account for 2-3% of the total number of childhood pre B-ALL [8]. Low hypodiploid karyotypes are also associated with a high frequency of mutations in *RB1* and *IKAROS* gene family member *IKZF2* (HELIOS) [7]. Non-tumor cells have *TP53* mutations in up to 43% of cases, indicating that the mutations are inherited and that LH-ALL is a manifestation of Li-Fraumeni syndrome [7]. Patients with *TP53* mutations acquired during leukemogenesis should not have detectable levels of *TP53* mutation after remission is achieved, whereas patients with germline *TP53* mutations will remain positive after remission. Although a deletion in the *TP53* gene was found after remission in our patient, consistent with an inherited mutation, testing of the patient's parents for *TP53* mutations was negative. The leukemic cells in our case showed a missense mutation affecting the codon described to code for the "hotspot" residue Arg175 [2] and loss of the other *TP53* allele due to chromosome 17 deletion. This pattern of genetic loss is consistent with the "two-hit" hypothesis, because biallelic alteration of a tumor suppressor gene is necessary for carcinogenesis. The aggressive clinical phenotype seen with loss of *TP53* function may be caused by loss of cell cycle arrest in G0/G1 phase and permitting passage through S phase [8]. The alterations of *TP53* gene are a hallmark of LH-ALL and the remarkably poor prognosis of LH-ALL might be due to high mutation frequency of *TP53* in this subset [4].

Development of a secondary malignancy following the treatment of childhood ALL has been variably reported to occur in between 1% to 10% of cases depending on the type of therapy used and the thoroughness of follow-up [9]. The most common types of secondary malignancy following treatment of ALL in children are acute myeloid leukemia, followed by MDS and nonmeningioma brain tumors [9]. In a search of the literature, we found one case of MDS following complete remission in a patient with *TP53* mutations found before and after remission that had received a stem cell transplant [8].

Therapy related MDS (t-MDS) occurs in patients exposed to genotoxic chemotherapy, irradiation or both. Cases are attributed to prior therapy based on circumstantial evidence, such as: 1) resistance of typical clonal cytogenetic abnormalities; 2) significant exposure to leukemogenic therapy (e.g., at least one cycle); and 3) sufficient latency from exposure to diagnosis of t-MDS (e.g., at least 6 months). All the criteria were met in our case. Regardless of the cause, t-MDS has an inferior prognosis compared to

de novo disease. Loss of material from chromosomes 5 and/or 7 is detectable in up to 70% of t-MDS patients, often with other abnormalities in a complex karyotype [10], such as in our case. Point mutations in *TP53* are more common in t-MDS compared to *de novo* MDS.

In conclusion, in patients diagnosed with LH-ALL, *TP53* gene analysis for mutations should be done routinely. The alterations of *TP53* gene are a hallmark of LH-ALL and the remarkably poor prognosis and high risk of treatment failure of LH-ALL might be due to high *TP53* mutation frequency in combination with loss of second *TP53* allele due to monosomy 17 [4]. We also favor that this entity, LH-ALL with concomitant *TP53* mutation, be carefully distinguished from other ploidy groups found in acute lymphoblastic leukemia [4]. Lastly, LH-ALL harboring *TP53* mutation should be carefully monitored as they may develop MDS in the near future.

Acknowledgements

The authors declare no conflicts of interests.

Received: December 17, 2015 **Accepted**: January 23, 2016
Published: March 1, 2016

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