Article published online: 2022-12-21

PAX5 and TDT-Negative B-Acute Lymphoblastic Leukemia with Unusual Genetic Mutations: A Case Report

Tariq N. Aladily[1] Jamil F. Qiqieh[1] Alaa Alshorman[2] Salem Alhyari[2] Majd Khader[3]

[1] Department of Hematopathology, The University of Jordan, Amman, Jordan
[2] Department of Hematology and Oncology, The University of Jordan, Amman, Jordan
[3] Department of Pathology, King Hussein Cancer Center, Amman, Jordan

Address for correspondence Tariq N. Aladily, MD, Department of Hematopathology, The University of Jordan, Amman 11814, Jordan (e-mail: tnaladily@ju.edu.jo).

Avicenna J Med 2022;12:186–190.

Abstract

B-acute lymphoblastic leukemia (B-ALL) is commonly encountered in clinical practice. Patients present with increased percentage of lymphoblasts in bone marrow and/or peripheral blood. Immunophenotypic study by flow cytometry or immunohistochemistry is essential to establish the diagnosis. Paired box-5 (PAX5) is a B cell lineage protein and terminal deoxynucleotidyl transferase (TDT) is an immature marker, both of which are routinely tested in the pathologic workup of acute leukemia. In this report, we describe a case of B-ALL in a 37-year-old woman in which both PAX5 and TDT were negative. Next-generation sequencing test detected mutations in DNA methyltransferase 3 α and Fms related receptor tyrosine kinase 3 genes, which are frequently mutated in acute myeloid leukemia rather than B-ALL. The constellation of these rare findings in a single case signifies the importance of examining a wide panel of markers when the diagnosis of ALL is suspected.

Keywords

- ALL
- B-ALL
- acute leukemia
- TDT
- PAX5
- immunohisto chemistry
- flow cytometry
- DNMT3A
- FLT3

Introduction

B-acute lymphoblastic leukemia (B-ALL) is an aggressive neoplasm of B-lymphoblasts that affects the bone marrow and peripheral blood. It is the most common childhood malignancy with an approximate incidence of 25% of malignancies in children younger than 15 years. Yet, it is also frequently encountered in adult group. The diagnosis of B-ALL essentially relies on the pathologic examination of morphology and immunophenotype of blasts.[1]

Terminal deoxynucleotidyl transferase (TDT) is an intranuclear DNA polymerase encoded by DNA nucleotidylexotransferase gene in the region 10q23-q24. It is responsible for inserting nucleotides at the V(H)-D and D-J(H) regions of immunoglobulin genes resulting in the diversity of immunoglobulin heavy chain. TDT is highly expressed in normal and transformed lymphoid precursor cells.[1]

Paired box-5 (PAX5) is a transcription factor protein that is essential for commitment of lymphoid progenitors to B cell lineage. The gene is located in chromosome 9p13 region and it is expressed in early, but not late stages of B cell differentiation.[2] In this report, we describe a case of B-ALL that was negative for both TDT and PAX5, representing a diagnostic challenge and highlighting the importance of applying a wider panel of immunophenotypic testing in diagnostic hematopathology.

DOI https://doi.org/10.1055/s-0042-1758387.
ISSN 2231-0770.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

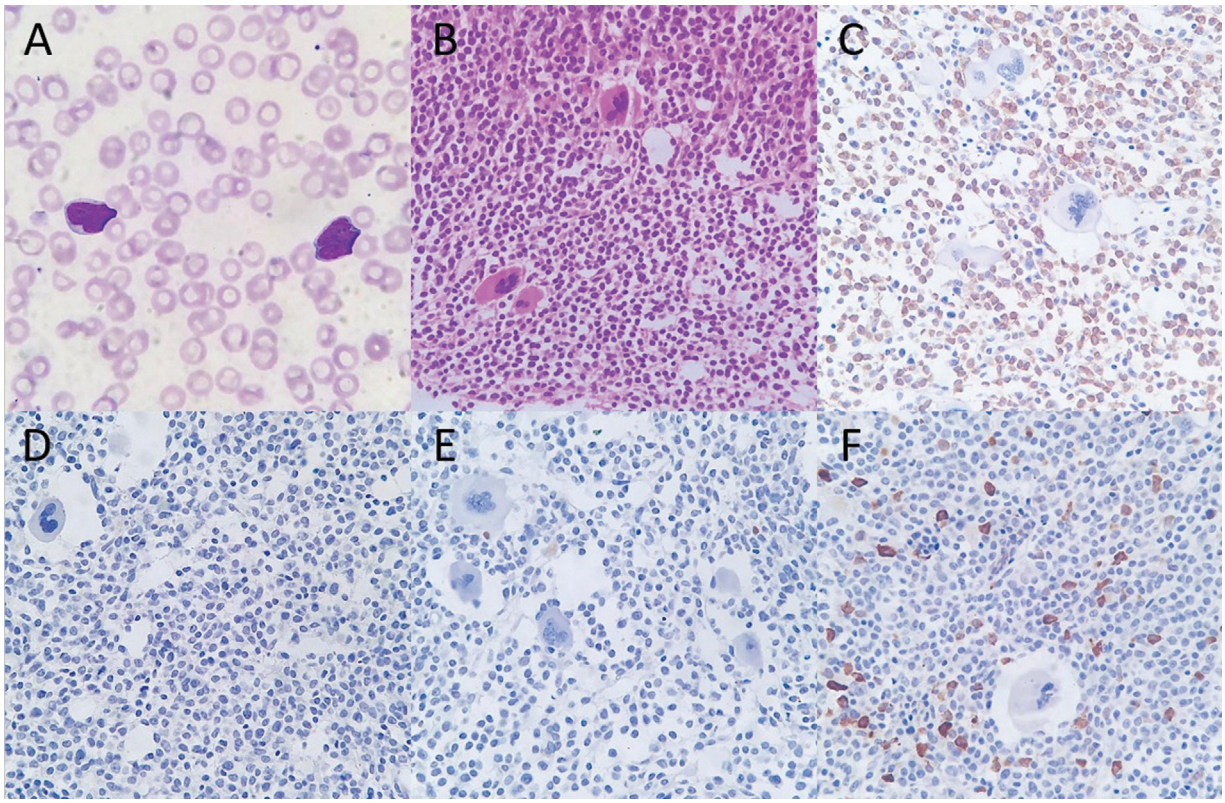

Fig. 1 (**A**) Peripheral blood smear from the patient showed numerous lymphoblasts with characteristic minimal agranular cytoplasm along with irregular nuclear contour, fine chromatin and small nucleoli (Leishman stain, 600X). (**B**) Bone marrow core biopsy revealed sheets of small blasts effacing most of hematopoietic cells (hematoxylin and eosin, 400X). The blasts were positive for (**C**) CD34 and negative for (**D**) terminal deoxynucleotidyl transferase, (**E**) paired box-5, and (**F**) myeloperoxidase (hematoxylin counter stain, 400X).

Case Report

A 37-year-old lady presented to the hematology clinic complaining of a 1-month history of easy fatiguability, feeling of hotness without a documented fever, loss of appetite, unintentional weight loss, and abdominal pain. Physical examination revealed pallor, yellowish discoloration of sclera and skin and widespread nonblanchable petechial rash over her body. Abdominal examination demonstrated right upper quadrant tenderness and hepatosplenomegaly.

Computed tomography scan of the chest, abdomen, and pelvis showed small axillary lymph nodes measuring 7 mm in short-axis along with few scattered pulmonary nodules. Hepatomegaly (liver span of 18 cm) without a definite focal lesion was evident, as well as splenomegaly with wedge-shaped irregular hypodensities and minimal amount of pelvic free-fluid.

Laboratory tests showed elevated serum alanine and aspartate aminotransferases (7.7 and 4.7 µKat/L, respectively), direct bilirubin (8.6 µmol/L), and blood urea nitrogen (10.7 mmol/L). Complete blood count showed pancytopenia: hemoglobin concentration of 66 g/L, white blood cell (WBC) count of 0.72×10^9, and platelet count of 86×10^9. Blood film examination revealed 82% blasts of all the nucleated cell (►Fig. 1A). The patient underwent a bone marrow biopsy. Bone marrow smears showed abundant blasts of similar morphology to the peripheral blood. Bone marrow core biopsy demonstrated hypercellular marrow spaces where hematopoietic cells were replaced by sheets of small blasts occupying approximately 80% of total cells (►Fig. 1B). Immunohistochemical stain study showed the blasts were positive for CD34 and negative for PAX5, TDT, myeloperoxidase (MPO), CD3, lysozyme, CD4, CD43, CD61, and CD71 (►Fig. 1C–F). A provisional diagnosis of acute undifferentiated leukemia was made. Flow cytometry (FC) study on bone marrow aspirate showed the blasts were positive for CD45 (dim), CD34, HLA-DR, CD19, CD79a, CD38, CD10 (subset), CD117 (subset), CD56 (subset) and CD123, and negative for TDT, MPO, CD20, cytoplasmic (cy) CD3, surface (s) CD3, CD4, CD14, CD64, CD33, CD13, CD15, CD5, CD7, CD2, and CD8 (►Fig. 2). The diagnosis of B-ALL was established. A verbal consent was taken from patient to publish the case.

Conventional cytogenetic study showed a complex karyotype including chromosomes 12, 13, and 19 and derivative chromosome 3 with inversion and deletion in the long arm: (der(3)del(3)(q12q24) inv(3)(q25q27), t(12:13:19)(q22:q21:913.1) [13]/49,XX). Fluorescence in situ hybridization test demonstrated deletion in chromosome 3 involving a segment that contains RPN1 gene. Next-generation sequencing test detected mutations in DNA methyltransferase 3 α (DNMT3A) and Fms related receptor tyrosine kinase-3 (FLT3) genes (exon 23, nucleotide: c.2645G > A, exon 14, nucleotide: c.1792_1793ins, respectively).

The patient was started on chemotherapy. She received cyclophosphamide, vincristine, doxorubicin, dexamethasone (hyper-CVAD), and L-asparaginase regimen. After 3 weeks, a follow-up bone marrow biopsy was performed

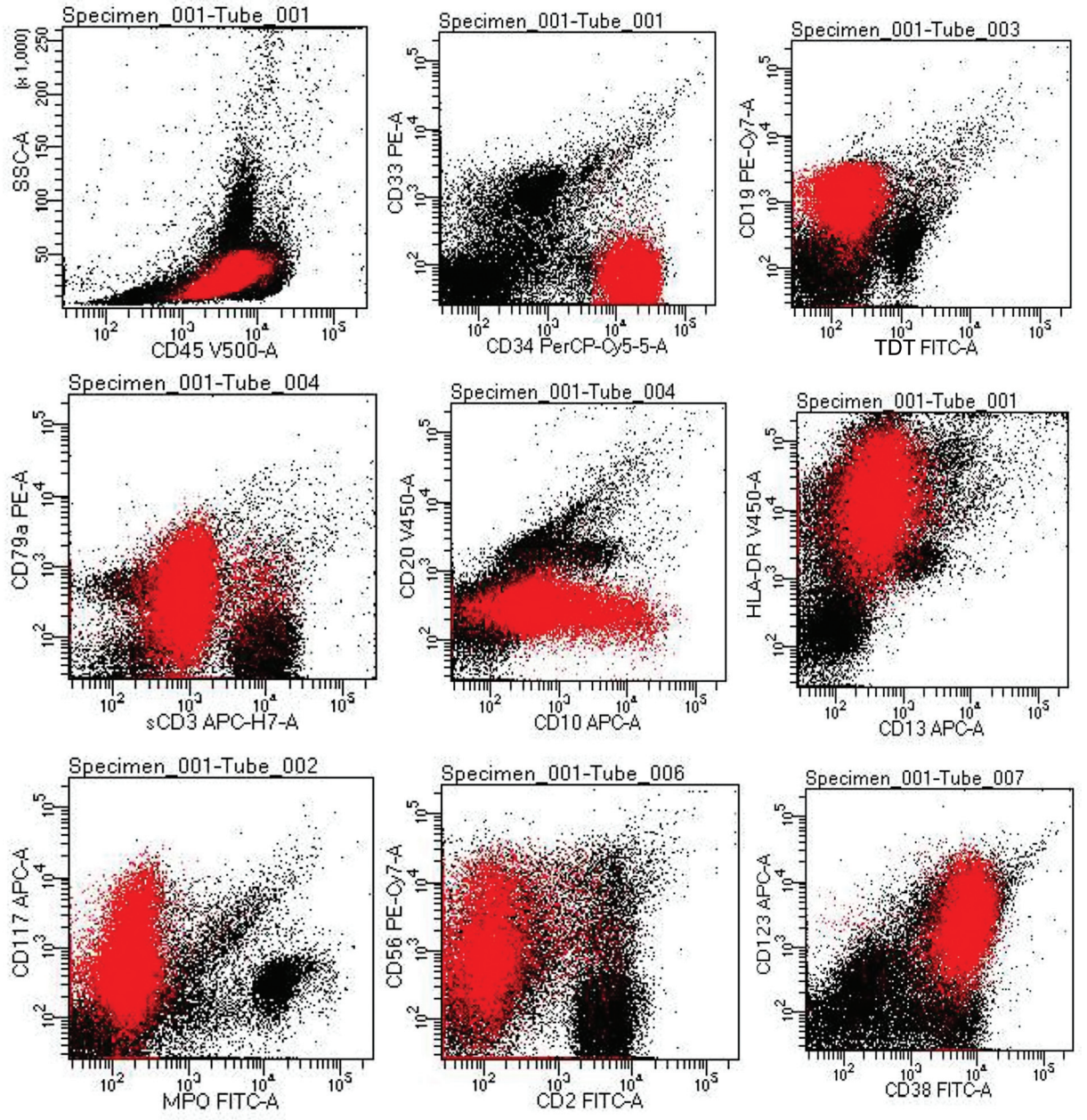

Fig. 2 Multicolor flow cytometry study from bone marrow aspirate. Gating strategy focused on the population of CD45-dim and low side-scatter where blasts normally appear. The population was positive for CD34, CD19, CD79a (subset), CD10 (subset), HLA0DR (bright), CD117 (subset), CD56 (subset), CD123, and CD38, while negative for CD33, terminal deoxynucleotidyl transferase (TDT), (s)CD3, CD20, CD13, myeloperoxidase (MPO), and CD2.

to examine the response status to initial chemotherapy, but it showed a persistent disease of 45% blasts with a similar morphology to previous biopsy. The patient decided to receive alternative medicine and to stop chemotherapy and she signed against medical advice. Two months later, she was admitted to the hospital for orbital cellulitis that was complicated by sepsis. The patient passed away 6 days later despite supportive management.

Discussion

B-ALL is a malignant neoplasm that shows impaired maturation and increased proliferation of precursor B-cells. It is the second most common acute leukemia in adults comprising three-fourth of cases of ALL, while the remainder is of T cell origin. Compared with pediatric group, the disease is less common in adults and it shows a more aggressive clinical course and a worse prognosis. The long-term remission does not exceed 40% of cases despite high-response rate to initial induction therapy. Poor prognostic factors include old age, high WBC count at presentation, the presence of residual disease after initial therapy, and certain cytogenetic aberrations such as Philadelphia chromosome, hypodiploidy, complex karyotype, t(4;11), t(8;14), and Philadelphia-like genomics.[3]

The diagnosis of B-ALL is established when lymphoblasts count in increased, usually above 25% of nucleated cell in

bone marrow or peripheral blood. The B cell lineage is characterized by expression of CD19, (c)CD79a, and/or (c) CD22, while TDT, CD10, CD38, and/or CD34 positivity determine the immature nature of these cells. Most B-ALL cases are also positive for CD10. By tissue sections, PAX5 is frequently used by hematopathologists and is considered the most specific marker of B cell differentiation, while CD20 expression is variable. The myeloid markers of CD13, CD33, and CD117 can be rarely expressed.[3] The diagnosis of B-ALL is straightforward in most patients; however, some cases carry an atypical immunophenotype making the diagnosis challenging. The B-lymphoblasts in the patient we describe were negative for both TDT and PAX5, which may be confused with another mature and nonlymphoid type of leukemia.

Cases of B-ALL that are truly negative for TDT were described in the literature. In two studies, the frequency of TDT-negative among a series of B-ALL ranged between 2.5 and 11%, where it was evaluated by both FC and immunohistochemistry (IHC).[2,4,5] Another larger study of 917 patients demonstrated a TDT-negative rate of 2.3%, which was examined by FC only.[6] The authors found that TDT-negative patients tended to be younger, had high WBC count, and carried KMT2A-r mutation. However, the 5-year overall survival was not different from TDT-positive patients. Of note, the patients in those studies were mostly children, necessitating the exclusion of Burkitt leukemia. Thus, TDT-negativity by itself does not exclude the diagnosis of B-ALL and examining other immature markers such as CD34, HLA-DR and the absence of light-chains/immunoglobulins is essential to reach the correct diagnosis.

PAX5 is a specific marker for B cell lineage that is commonly examined by IHC. The sensitivity varied among studies in the literature and ranged between 72 and 100% of B-ALL cases.[2,7] A previous study showed that PAX5 gene is commonly deleted or translocated in B-ALL in both children and adults.[8] However, negative expression of PAX5 by IHC does not reflect the actual status of genetic mutation. It was found that PAX5 is predominantly expressed by one of its two alleles. If genetic mutation affects the non-functional allele, then its protein expression will not be affected. On the other hand, genetic deletion of the functional allele is not compensated and thus will result in a decreased protein production. Clinically, the patients' outcome appeared not to be affected by genetic mutation or protein absence.[9] The patient we described was negative for PAX5 and CD20 by IHC. Thus, using a short panel may miss the B-nature of these cells and render an erroneous diagnosis.

DNMT3A gene encodes an epigenetic regulator that is responsible for methylation of CpG dinucleotides. It is commonly mutated in acute myeloid leukemia along with FLT3 gene and is associated with adverse outcome. The incidence of these mutations is B-ALL appears to be rare, accounting for 1.4 and 5%, respectively.[9,10] Amplicon-targeted next-generation sequencing showed a distinct pattern of FLT3 mutation in B-ALL as it is characterized by juxtamembrane insertion and deletion or juxtamembrane point mutations. Patients with FLT3-positive B-ALL were found to have frequent disease relapse and poor prognosis, with a potential response to tyrosine kinase inhibitors therapy

Conclusions

In short, we describe a case of B-ALL with multiple unusual immunophenotypic and genetic findings. The negativity for TDT and PAX5 in leukemia workup may indicate an alternative diagnosis of myeloid or a mature nature of cells. It is imperative to examine the full panel of acute leukemia immunophenotype and to correlate IHC with FC results. In addition, DNMT3A and FLT3 genetic mutations may still appear in B-ALL and do not, by default, indicate myeloid origin of blasts. The poor outcome in our patient may be secondary to the presence of complex karyotype and to poor compliance to chemotherapy rather than to the anomalous immunophenotype.

Funding

None.

Conflict of Interest

None declared.

References

1 Yasmeen S, Rajkumar A, Grossman H, Szallasi A. Terminal deoxynucleotidyl transferase (TdT)-negative lymphoblastic leukemia in pediatric patients: incidence and clinical significance. Pediatr Dev Pathol 2017;20(06):463–468
2 Kim M, Choi JE, She CJ, et al. PAX5 deletion is common and concurrently occurs with CDKN2A deletion in B-lineage acute lymphoblastic leukemia. Blood Cells Mol Dis 2011;47(01):62–66
3 Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J 2017;7 (06):e577
4 Liu L, McGavran L, Lovell MA, et al. Nonpositive terminal deoxynucleotidyl transferase in pediatric precursor B-lymphoblastic leukemia. Am J Clin Pathol 2004;121(06):810–815
5 Jamal S, Meraj F, Mansoor N, Parveen S, Shaikh A, Jabbar N. Distribution of subtypes and immunophenotypic characterization of 1379 cases of paediatric acute leukaemia. Pak J Med Sci 2021;37(03):805–811
6 Klairmont MM, Zhou Y, Cheng C, et al. Clinicopathologic and prognostic features of TdT-negative pediatric B-lymphoblastic leukemia. Mod Pathol 2021;34(11):2050–2054
7 El MA, ElSakhawy YN, Sallam MT. The value of anti-Pax-5 immunostaining in pediatric acute leukemia. Egypt J Haematol 2014;39(01):32
8 Nasr MR, Rosenthal N, Syrbu S. Expression profiling of transcription factors in B- or T-acute lymphoblastic leukemia/lymphoma and Burkitt lymphoma: usefulness of PAX5 immunostaining as pan-Pre-B-cell marker. Am J Clin Pathol 2010;133(01):41–48
9 Kim MS, Kim YR, Yoo NJ, Lee SH. Mutational analysis of DNMT3A gene in acute leukemias and common solid cancers. APMIS 2013; 121(02):85–94
10 Zhang Y, Zhang Y, Wang F, et al. The mutational spectrum of FLT3 gene in acute lymphoblastic leukemia is different from acute myeloid leukemia. Cancer Gene Ther 2020;27(1-2):81–88

