CASE REPORT

FLI1 and MIC2 expression in precursor B-lymphoblastic leukemia with Burkitt-like morphology and extensive extramedullary involvement: A diagnostic challenge in pediatric small round cell tumor

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ABSTRACT

Pediatric small round cell tumors (PSRCTs) constitute a large proportion of childhood malignancies with overlapping diagnostic and clinical features but radically different therapies. Here, we report a case of 16-year-old male child presenting with diffuse abdominal and mediastinal mass, axillary lymphadenopathy, and pleural effusion. Bone marrow aspirate showed near total replacement by small round malignant cells. The bone marrow biopsy showed interstitial infiltration by malignant cells, which were CD45– CD3– CD20– MIC2+ FLI1+ and diagnosis of Ewing's sarcoma was established. In contrast, flowcytometric immunophenotyping of the bone marrow aspirate showed CD45– cells, which were CD19+ cytCD79a+ CD10+ CD81+ CD38+ HLA-DR+ CD22+ CD20– consistent with B-cell acute lymphoblastic leukemia (B-ALL). The extended immunostaining panel on bone marrow biopsy also showed positivity for cytCD79a, CD10, CD19, and BCL-2, whereas fluorescent in-situ hybridization for EWSR1 gene rearrangement was negative. Thus, a final diagnosis of CD45– FLI1+ MIC2+ B-ALL was established. Rare cases of CD45– B-ALL with immunoreactivity for MIC2 and Friend leukemia virus integration 1 (FLI1) have posed a diagnostic challenge for PSRCTs in the recent past. This case report highlights the role of multimodality approach in establishing a correct diagnosis in CD45– PSRCTs to ensure definitive therapy and better clinical outcome.

KEY WORDS: B-ALL, FLI1, MIC2, multiparametric flow cytometry, pediatric small round cell tumors (PSRCTs)

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INTRODUCTION

Pediatric small round cell tumors (PSRCTs) constitute a major fraction of childhood malignancies with overlapping morphology and clinical presentation wherein precise and accurate diagnosis is required because of completely different treatment protocols. The important differentials for PSRCTs presenting with extensive mediastinal and abdominal involvement include Primitive Neuro-Ectodermal tumor (PNET)/Ewing's sarcoma and non-Hodgkin lymphoma (NHL). The differentiation between these two entities (PNET/Ewing's vs. NHL) is based on histopathological and immunohistochemical evaluation with PNET/Ewing's being CD45–, MIC2 (CD99), and Friend leukemia virus integration 1 (FLI1) positive while the NHL is CD45+ MIC2– FLI1–. To add to this conundrum, a few cases of CD45– B-ALL with immunoreactivity for MIC2 and FLI1 have been reported in the recent past posing a diagnostic challenge for PSRCTs.[1]

CASE HISTORY

A 16-year-old male presented with 3 months history of fever, breathlessness, loss of weight, and appetite. On examination,

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pallor and pedal edema was present along with left axillary lymphadenopathy and a diffuse abdominal mass. Hemogram showed Hb of 9.4 g/dL, Total Leucocyte Count of 9.3 × 10^9/L, and platelet count of 179 × 10^9/L. Biochemical parameters showed markedly elevated Lactate Dehydrogenase (2116 IU/L) and C-Reactive Protein (67.3 mg/L). Kidney function tests and liver function tests were within normal limits. Peripheral blood smear examination was unremarkable except for the presence of neutrophilia. Contrast-enhanced computed tomogram revealed a bulky soft tissue mass in the anterior mediastinum with mild-to-moderate contrast enhancement, irregular contours, vascular encasement, mediastinal lymphadenopathy, and right-sided pleural effusion. Retroperitoneal lymphadenopathy with deposits in both kidneys was also noted. The radiological diagnosis was PNET or non-Hodgkin's lymphoma.

Bone marrow aspirate showed near total replacement by small round cells, slightly larger than the size of lymphocytes with deeply basophilic scant cytoplasm, and non-coalescing vacuoles [Figure 1]. The bone marrow biopsy showed interstitial infiltration by malignant round cells, which on immunohistochemistry were reactive for MIC2 (CD99) and FLI1 and negative for CD45, CD3, CD20, and desmin. The histopathological diagnosis of Ewing's sarcoma was established [Figure 2]. On flow cytometric immunophenotyping of the bone marrow aspirate, 35% CD45− cells were noted, which were CD19+ cyt CD79a+ CD10+ CD81+ CD38+ HLA-DR+ CD22+ and were negative for CD20, surface immunoglobulins (kappa-, lambda-, ImmunoGlobulinM-), myeloid markers (cytMPO−, CD13−, CD33−, CD117−), and T-cell markers (CD3−, CD7−, CD2−) [Figure 3]. A diagnosis of B-ALL with a differential diagnosis of Burkitt's leukemia with atypical immunophenotype was considered. With two discrepant diagnosis of B-ALL and Ewing's sarcoma, a state of great diagnostic dilemma existed as treatment of both the conditions is totally different. At this stage, bone marrow biopsy was evaluated for the expression of additional immunomarkers.

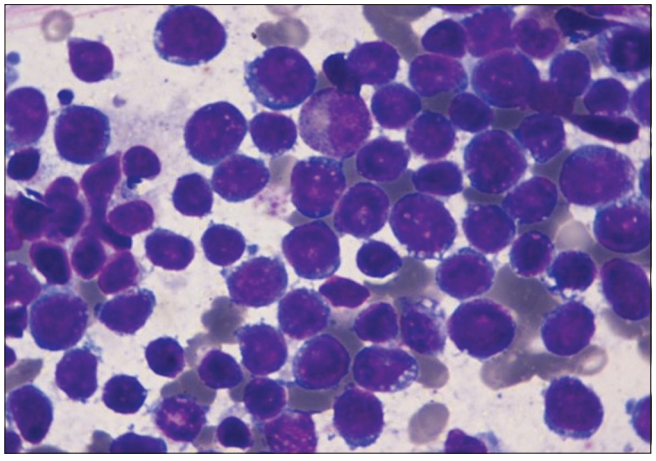

Figure 1: Morphological characteristics of bone marrow aspirate (MGG staining, 100×): showing infiltration by intermediate sized cells with dense hyperchromatic nuclei, deeply basophilic cytoplasm, and multiple non-coalescing vacuolations (MGG: May Grunwald Giemsa)

An axillary lymph node biopsy was also done to rule out the possibility of synchronous malignancies. The extended immunostaining panel on bone marrow biopsy showed the tumor cells to be positive for CD79a, CD10, CD19, and BCL-2 [Supplementary Figure 1]. The lymph node biopsy showed complete effacement of nodal architecture by intermediate sized cells which were Tdt+ CD79a+ CD10+ BCL-2+ FLI1+ MIC2+ [Supplementary Figure 2] and negative for CD45, CD3, CD20, CD2, CD5, cyclinD1, and BCL-6. Conventional chromosomal analysis (Giemsa banding) revealed no evidence of any structural or numerical abnormality. Fluorescent in-situ hybridization (FISH) for EWSR1 gene rearrangement was negative.

Thus, a final diagnosis of CD45− FLI1+ MIC2+ B-ALL was established. Patient was treated with BFM-90 protocol for B-ALL. A repeat hematological evaluation at day 21 showed normalization of hematological parameters. Minimal residual disease assessment by multiparametric flowcytometry was negative for leukemic cells. Thus, the patient responded well to B-ALL induction therapy.

DISCUSSION

Ninety percent of B-ALL cases predominantly present as pure leukemia and the remaining 10% as lymphoblastic lymphoma with an isolated extramedullary disease and limited peripheral blood and bone marrow involvement.[2,3] In the index case, despite extensive involvement of mediastinum and abdominal organs and lymph nodes, hemogram and peripheral blood parameters were near normal and this restricted clinical differential diagnosis to Ewing's/PNET and lymphoblastic lymphoma. Aleukemic presentation of B-LBL often mimics other round cell tumors in pediatric patients, and in the absence of CD45 positivity, it can be easily misdiagnosed as non-hematopoietic round cell tumor,

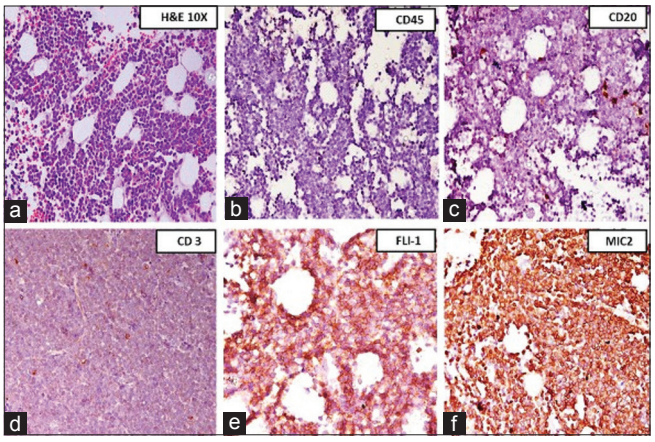

Figure 2: H and E staining of bone marrow biopsy showing diffuse infiltration by small round cell tumor with dense hyperchromatic nuclei, inconspicuous nucleoli, and scant cytoplasm (a), immunohistochemistry with antibodies against indicated markers including CD45− (b), CD20− (c), CD3− (d), MIC2+ (e), and FLI1+ (f) (H and E: hematoxylin and eosin)

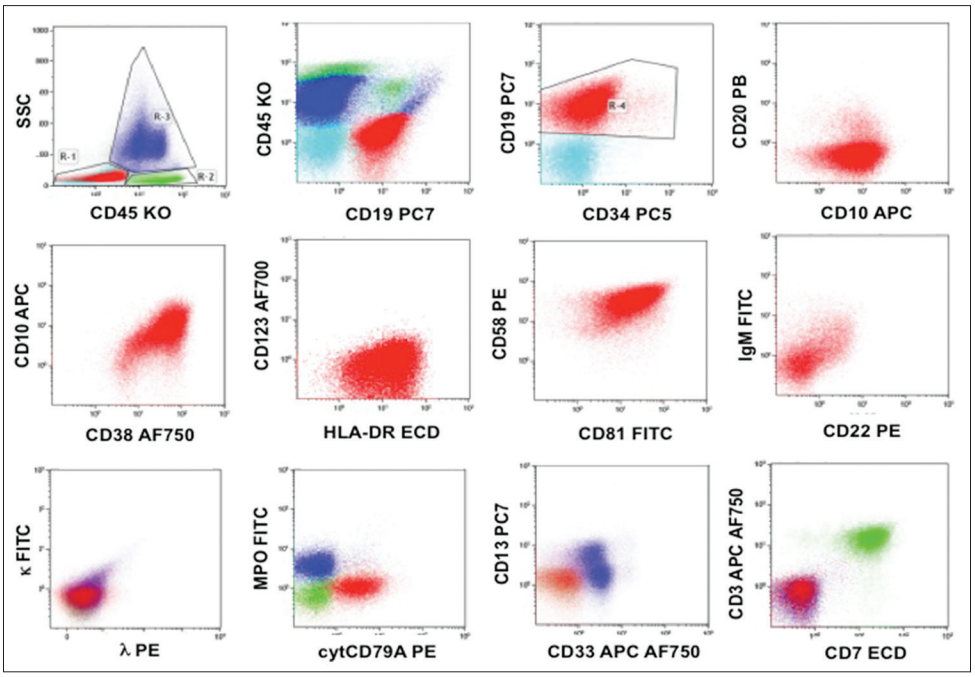

Figure 3: Flow cytometric immunophenotyping of bone marrow aspirate: Cells were stained with fluorescently conjugated antibodies, 2 tubes – 10 color panel. Shown are dot plots as indicated which included CD45– blasts (R1), CD19+, Cyt79a+ , CD34–, CD38+, CD10+, CD20–, HLA-DR+, CD123–, CD22–, SIgM–, CD13–, CD33–, cytMPO–, and cyt 3–

most commonly PNET/Ewing's. Interestingly in our patient, both FLI1 and MIC2 were positive, which was almost consistent with Ewing's sarcoma considering clinical presentation, uninvolved peripheral blood, and CD45 negativity in tumor cells.

Expression of FLI1 is associated with malignant transformation in various non-hematopoietic malignancies; FLI1 is a transcription factor, which was first identified as a proto-oncogene in Friend virus-induced erythroleukemias.[4] It is highly expressed in hematopoietic cells and plays a critical role in normal hematopoiesis.[5] Ewing's sarcoma is the most common malignancy involving FLI1 as gene product of [t(11;22)(q24;q12)] and the EWS/FLI1 fusion protein.[6] However, FLI1 positivity is also seen in hematopoietic malignancies and is a major diagnostic pitfall in leukocyte common antigen (LCA)-negative pediatric round cell tumors. Studies have shown that the absence of LCA immunoreactivity does not exclude ALL and FLI1 positivity is not restricted to Ewing sarcoma.[1] Similarly, MIC2 expression is not restricted to PNET/Ewing's sarcoma and has been reported in pediatric B-ALL/LBL.[7]

In our case, immunostaining of bone marrow biopsy with an extended antibody panel distinctly showed the reactivity for cytCD79a, TdT, and CD19, which was not considered earlier because of FLI1 and MIC2 positivity in a CD45– case. Similar pattern of immunoreactivity was observed on axillary lymph node biopsy as seen in bone marrow biopsy. Flowcytometric immunophenotypic analysis on bone marrow aspirate also showed B-ALL phenotype in CD45– blasts. Taken together, our observations suggest that B-ALL should not be ruled out based on CD45 and CD20 negativity in PSRC. For an accurate and precise opinion, a comprehensive B-cell panel must be included. As far as MIC2 and FLI1 reactivity is concerned, these act as masquerder in this case as the possibility for PNET/Ewing's was ruled out by EWSR1 negativity by FISH.

CONCLUSION

A multidisciplinary approach involving morphology, comprehensive antibodies panel, cytogenetic, and molecular studies must be considered in all PSRCTs before deriving an accurate diagnosis for timely institution of definitive therapy.

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Conflicts of interest
The authors declare no competing or conflict of interests.

REFERENCES

1. Lin O, Filippa DA, Teruya-Feldstein J. Immunohistochemical evaluation of FLI1 in Acute Lymphoblastic Lymphoma (ALL): A potential diagnostic pitfall. Appl Immunohistochem Mol Morphol 2009;17:409-12.
2. Maitra A, McKenna RW, Weinberg AG, Schneider NR, Kroft SH. Precursor B-cell lymphoblastic lymphoma. A study of nine cases lacking blood and bone marrow involvement and review of the literature. Am J Clin Pathol 2001;115:868-75.
3. Chen CC, Weng HH, Hwang CE, Lu CH, Chen PT, Gau JP. Acute leukemia presenting with extramedullary diseases and completely normal hemogram: An extremely unusual manifestation unique to pre-B ALL. Am J Hematol 2010;85:729-31.
4. Ben-David Y, Giddens EB, Bernstein A. Identification and mapping

of a common proviral integration site FLI1 in erythroleukemia cells induced by Friend murine leukemia virus. Proc Natl Acad Sci U S A 1990;87:1332-6.
5. Truong AH, Ben-David Y. The role of FLI1 in normal cell function and malignant transformation. Oncogene 2000;19:6482-9.
6. Bailly R, Bosselut R, Zucman J, Cormier F, Delattre O, Roussel M, *et al*. DNA-binding and transcriptional activation properties of the EWS-FLI1 fusion protein resulting from the t(11;22) translocation in Ewing sarcoma. Mol Cell Biol 1994;14:3230-41.
7. Cox CV, Diamanti P, Blair A. Assessing CD97 and CD99 as markers of leukaemia initiating cells in paediatric ALL. Blood 2012;120:1882.