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CASE REPORT

Mixed phenotypic acute leukemia (B/T), with t(9;22)(q34;q11.2);BCR-ABL1: a rare phenomenon and strange phenotype

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Abstract Mixed phenotypic acute leukemia (MPAL) is an uncommon hematological neoplasm which can either contain distinct blast populations, each of a different lineage (bilineal) or one population with multiple antigens of different lineage on the same cells (biphenotypic). t(9;22)(q34;q11.2;BCR-ABL1) is the most common recurrent genetic abnormality in MPAL; however, it is a very rare leukemia that accounts for less than 1 % of all acute leukemias and is usually associated with poor outcome. Common phenotypic expression of MPAL includes T/Myeloid and B/Myeloid. Leukemic blast showing evidence of both B and T lineage commitment has been described as a very rare phenomenon in the existing literature. We here report a rare case of MPAL (B/T lineage) with t(9;22)(q34;q11.2;BCR-ABL1) along with morphologic and immunophenotypic findings with special emphasis on need to differentiate such cases from patients with CML-Blast crisis as therapy, and prognosis in these two scenarios differ significantly.

Keywords Mixed phenotypic acute leukemia · Biphenotypic · Immunophenotyping

Introduction

Mixed phenotypic acute leukemia (MPAL) is a heterogenous and uncommon hematological disorder which comprises approximately 2–5 % of all acute leukemias [1]. Two main recurrent genetic abnormalities seen in MPAL are t(9;22)(q34;q11) *BCR-ABL1* and t (v;11q23), found frequently enough in these cases to be considered as separate entities in recent WHO (2008) classification. t(9;22)(q34;q11.2);BCR-ABL1 represents the most common abnormality in MPALs; however, it is a rare entity, accounting for less than 1 % of all leukemias [2].

In the absence of these genetic abnormalities, MPAL is subtyped based on lineage of blasts. Immunophenotypically, four possible combinations have been reported [3]. Most frequent among these is B/Myeloid followed by T/Myeloid. Rarely combined B/T lineage has been reported; triphenotypic B/T/Myeloid being exceptional.

We here present a rare case of MPAL with t(9;22)(q34;q11.2);BCR-ABL1 and unusual B/T immunophenotype along with aberrant expression of CD13, CD33, and CD56. Combined B/T immunophenotype is a very rare and unusual phenomenon in MPAL. To the best of our knowledge, only four cases of B/T acute biphenotypic leukemia have been reported in the literature before; the present case represents the fifth one.

Case report

A 54-year-old gentleman presented to our medical out-patient clinic with frequent attacks of giddiness. There was no lymphadenopathy or hepatosplenomegaly. Complete hemogram revealed Hb 81 g/l, total leukocyte count 74×10^9 cells/l, and platelet count 79×10^9 cells/l. Peripheral smear examination shows 71 % circulating blasts. There was an admixture of smaller and larger blasts (60 and 40 %, respectively). Larger blasts were 2–3 times the size of small mature lymphocyte, have moderate amount of cytoplasm, dispersed chromatin, and prominent 1–2 nucleoli. Smaller blasts had scant

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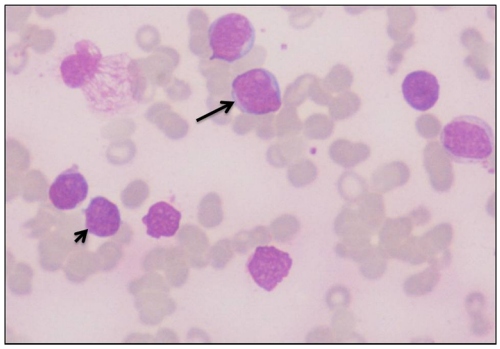

Fig. 1 Peripheral smear showing admixture of large (*long arrow*) and small (*short arrow*) blats (Leishman stain, ×400)

cytoplasm, condensed chromatin, and inconspicuous nucleoli (Fig. 1). Auer rods were not seen. All the blasts were negative for both myeloperoxidase (MPO) and periodic acid Schiff (PAS) cytochemical stains. Immunophenotyping (IPT) was carried out on 4-color BD FACS Calibur using standard protocol with panel of antibodies comprising of CD45, CD34, CD38, HLA-DR, Tdt, CD19, sCD22, cytCD22, CD10, CD2, CD3, cytCD3, CD5, CD7, CD13, CD33, CD117, CD14, and CD56. FSC/SSC and CD45/SSC gating strategies were used. The gated population of blasts showed bright expression for HLADR and CD19; moderate expression for CD10, CD34, cytCD3, and CD13; and dim expression for Tdt, CD22, cytCD22, sCD3, CD5, CD7, CD56, and CD33 (Fig. 2a–k). Markers found to be negative in blast population were CD2, sCD3, CD14, CD38, and CD117. IPT profile rendered a diagnosis of MPAL B/T lineage with aberrant expression of CD13, CD33, and CD56.

FISH performed using Vysis LSI dual color, dual fusion probes for BCR-ABL1 and dual color break-apart rearrangement probes for MLL revealed blasts cells positive for BCR-ABL1 gene rearrangement (Fig. 2l) and negative for MLL gene rearrangement. Peripheral blood sample was also subjected to cytogenetics to look for additional chromosomal abnormalities; however, no analyzable metaphases could be seen.

Discussion

Using currently available diagnostic tools, majority of acute leukemias can be subclassified into myeloid, B-lymphoid, or T-lymphoid lineage. However, lineage assignment is not possible in a minority of cases despite integration of data from flow cytometric, immunohistochemical, cytochemical, cytogenetic, and molecular genetic investigations [4, 5]. These cases have been designated as "acute leukemia of ambiguous lineage" by recent WHO classification. MPAL represents a subgroup of this entity where there is evidence of expression of both myeloid and lymphoid lineage specific antigens in the blast cells.

Before WHO (2008) revised the criteria for diagnosis of MPAL, the European Group for Immunological Classification

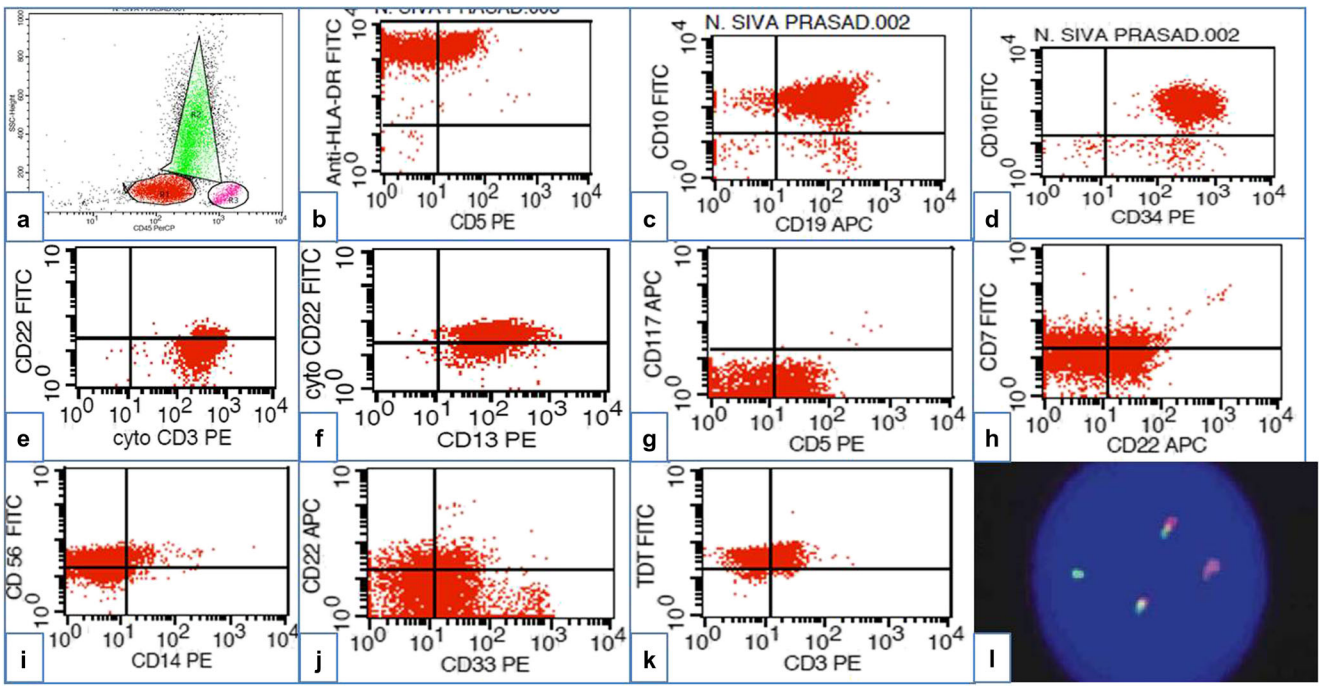

Fig. 2 **a**–**k** Scatterogram revealing B/T lineage phenotype: the gated population of cell showing bright expression for HLA-DR, CD19: moderate expression for CD10, CD34, cytCD3, CD13: dim expression for Tdt, CD22, cytCD22, sCD3, CD5, CD7, CD56, and CD33. **l** FISH performed using Vysis LSI dual color, dual fusion probes revealing blast cells positive for BCR-ABL gene arrangement

of Leukemias (EGIL 1998) criteria was widely adopted; however, it had its own limitations like B-lineage markers sometimes being expressed in AML with t(8;21)(q22;q22), T-lineage markers being present in few cases of AML with t(15;17)(q22;q12), and MPO reported to be present in 23 % of adult B-ALL [6]. Also, intensity of the expression of markers was not taken into consideration which is important for the diagnosis and subtyping of acute leukemias. Thus, using EGIL criteria might lead to misdiagnosis and overestimation of the prevalence of MPAL cases [7]. The current WHO (2008) classification, however, has dismissed these scoring systems and relies upon lineage assignment based on specific features [2].

Using recent WHO criterion, prevalence of MPAL has been found to be 1.6 % of acute leukemia [6]. Out of various reported immunophenotypic combination, MPAL B/T with blasts expressing both B- and T-lymphoid antigens is a very rare phenomenon with very few cases reported in the literature before. In our case, blasts expressed lineage-specific markers of B- and T-lineage. Expression of myeloid markers (CD13, CD33) and CD56 was also seen; however, MPO which is essential for assigning myeloid lineage to a blast population as per WHO (2008) classification was lacking.

Leukemogenesis of MPAL remains unclear as to whether it arises from a normal pleuripotent stem cell or because of severe genetic aberrations. The most frequent cytogenetic finding in MPAL is the Philadelphia chromosome, *BCR-ABL1* gene rearrangement which is also observed in chronic myeloid leukemia, B-precursor ALL (often expressing myeloid antigens but not fulfilling MPAL criteria), and in some cases of de novo AML pointing toward such possible phenotypic plasticity. Our case was found to have recurrent genetic abnormality t(9;22)(q34;q11.2);BCR-ABL1 and B/T immunophenotype with aberrant expressions of CD13, CD33, and CD56 which is a very unusual profile in these cases and may be explained by similar mechanism of phenotypic plasticity as mentioned above.

B/T MPAL as such is a very rare entity and represents less than 4 % of all MPAL [8]. Most of the cases of B/T MPAL found in literature describe biphenotypic blasts rather than bilineal blasts. In a study conducted by Weir et al., out of 19 cases of MPAL over a 10-year period, none of the cases was reported to be of B/T combined lineage. Matutes et al. [3] reported four cases of B/T MPAL, all of them being biphenotypic similar to the present case. Blasts in all four cases had strong CD19 expression together with one or more B-lymphoid markers. TdT was positive in three cases and CD34 in one case. Three of four cases had abnormal karyotype: BCR-ABL, hyperdiploidy, and complex karyotype in one case each, while one case was found to have normal karyotype.

t(9;22)(q34;q11) is the most frequent cytogenetic alteration seen in MPAL. Some patients with chronic myeloid leukemia may develop or even present with a mixed blast phase meeting the criteria for MPAL; however, this diagnosis should not be made in patients known to have had CML. The present case was positive for BCR-ABL translocation with no preceding CML. If the patient has an antecedent history of CML, then a diagnosis of CML-blast crisis rather than MPAL with special note describing the mixed phenotype population of blasts should be rendered.

The optimal therapeutic approach to MPAL cases is still unclear. There is not enough data to suggest whether patients with biphenotypic leukemia fare better on induction regimes designed for ALL or AML. As a rule, outcome has been reported to be very poor in these cases [9].

Our patient was administered induction chemotherapy Berlin-Frankfurt-Munster (BFM) protocol, demonstrated good response with morphological remission seen on day 22 marrow, and is under regular follow-up.

Conflict of interest The authors declare that they have no conflicts of interests.

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