A Bi-Lineal Acute Leukemia (T/Myeloid, NOS) with Complex Cytogenetic Abnormalities

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Abstract We report an unusual case of bi-lineal mixed-phenotype acute leukemia (T/Myeloid, NOS) with complex cytogenetic abnormalities in a 2-year-old boy. Despite attaining complete remission with therapy, he succumbed to status epilepticus following febrile illness. Flow cytometry represents the current standard of care for the diagnosis of this malignancy and the approach adopted in our case is discussed.

Keywords Acute leukemia of ambiguous lineage · Mixed-phenotype · Flow cytometric immunophenotyping · Pediatric malignancies · Childhood cancer

Introduction

Mixed phenotype acute leukemias may be bi-phenotypic, i.e. containing a single population of blasts co-expressing markers of more than one hemopoietic cell lineage, or bi-lineal, i.e. containing separate populations of blasts of multiple lineages. The latter are often challenging to analyze flow cytometrically and, with the 2008 WHO classification, require cytogenetic and/or molecular genetic analyses for comprehensive classification.

Case Report

A 2-year-old previously healthy boy was referred to haematology when he had rapid-onset malaise and high-grade fever with generalized lymphadenopathy and hepatosplenomegaly. His two older siblings were healthy, and there was no family history of hematological malignancy nor any stigmata of inherited bone marrow failure on physical examination.

Thoracic imaging showed mediastinal widening due to enlarged lymph nodes and bilateral pleural effusion. Haemoglobin was 81 g/l, platelets $106 \times 10^9/l$ and TLC $21.4 \times 10^9/l$. The blood film showed 21 % blasts of dimorphic appearance: predominantly small with high N/C ratio, scanty cytoplasm, coarse nuclear chromatin without nucleoli as well as another population of larger blasts with more vesicular chromatin and prominent nucleoli (Fig. 1). Cytochemically, ~12 % blasts, mostly larger ones, were positive for myeloperoxidase. Periodic acid Schiff stain was non-contributory by virtue of being negative in both types of blasts.

Flow cytometric immunophenotyping revealed two distinct CD34-positive clusters in the SSC^{low} -CD45^{dim} blast region (gating strategy described in Fig. 2). One cluster was

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Fig. 1 Dimorphic blasts including small lymphoid-looking ones as well as larger forms with more vesicular chromatin (Wright Giemsa stain, 1000 $\times$). The bone marrow biopsy also shows marked blast heterogeneity (Haematoxylin and eosin, 400 $\times$)

comprised of smaller-sized T-lymphoblasts (expressing cytoplasmic and surface CD3 with surface CD1a, CD2, CD4, CD5, CD7, dim CD8 and CD10 and negative for B-lymphoid antigens). Another cluster was comprised of larger-sized myeloblasts (expressing surface CD13, CD33, CD117, ~15 % positive for cytoplasmic myeloperoxidase, and negative for monocytic markers and CD41a) (Figs. 3, 4). Conventional karyotyping on 20 metaphases showed that 12 (60 %) were normal (46XY) while 8 (40 %) had del(11)(q23). In addition, 4 of the latter 8 (20 % overall) also displayed del(5)(q32) while another one possessed the inv(7)(q22q36) abnormality (Fig. 5). Reverse transcriptase PCR for bcr-abl and tel-amll was negative. The final diagnosis was mixed phenotype acute leukemia (bi-lineal, T-myeloid type) with complex cytogenetic abnormalities.

CSF examination by cytopspin was normal and other stigmata of CNS disease were absent. He received ALL-BFM-95 protocol chemotherapy and attained complete

Fig. 2 The CD45-SSC plot (*top left*) showed a complex mixture of merging populations in the blast region. The CD34-FSC plot (*top right* and *bottom left*) was used for the primary gating. It revealed two distinct CD34-positive populations. The larger one (P1, red, 12.6 %) tracked back to the CD45 dimmer blasts with relatively higher side

scatter while the smaller blasts (P2, green, 12.5 %) had CD45 intensity intermediate to P1 and lymphocytes and displayed lower side scatter. For tubes without CD34, these two regions of their CD45-SSC plots (*bottom right*) were used to assess blast immunophenotypes. (Color figure online)

Fig. 3 The larger blasts (P1 in Fig. 1, red) express CD33, CD117, CD13 and CD15. The smaller blasts (P2 in Fig. 1, green) express CD5, CD7, CD2 and CD4. (Color figure online)

remission. However, he succumbed 2 months after diagnosis to status epilepticus following a very brief febrile illness at home before specialized medical attention could be sought, possibly from CNS leukemia or infection. No autopsy was performed.

Discussion

Bi-lineal acute leukemias are rare hybrid neoplasms characterized by two or more distinctive blast populations, each exhibiting an unequivocal, lineage-specific differentiation pattern. T-myeloid is among the more frequent combinations seen in these rare tumours [1]. The blast heterogeneity is best demonstrated by multi-parameter flow cytometry, but, as in our case, may also be apparent morphologically and cytogenetically. MLL gene rearrangements (involving the 11q23 locus) are commonly seen and the current WHO classification

[2] recognizes MLL-rearranged mixed phenotype acute leukemias as a distinct diagnostic category. However, MLL-deleted cases are specifically excluded from this group, thereby making our case fit better into the mixed phenotype acute leukaemia, T/Myeloid, NOS WHO category.

The disease entity portends a poor clinical outcome. Only 2 of the 16 patients described in the largest series till date achieved durable disease-free remissions, although the type of chemotherapy administered was not mentioned [1]. On the other hand, the St. Jude's Children's Research Hospital [3] reported that 12/35 of their mixed phenotype acute leukemia patients (bi-phenotypic and bi-lineal) treated with ALL protocols had an 83 % CR rate as compared to a 61 % CR/PR rate in the 23/35 treated initially with an AML protocol. The two bi-lineal leukemias in the St. Jude study survived for 0.1 and 0.4 years respectively [3].

In conclusion, this case of a bi-lineal acute leukemia with complex cytogenetic abnormalities represents a rare,

Fig. 4 The larger blasts (P1 in Fig. 1, red) express CD13, HLA-DR and dim CD58. The smaller blasts (P2 in Fig. 1, green) express cytoplasmic CD3, surface CD1a and dim surface CD3. Both types of blasts express CD38. (Color figure online)

Fig. 5 Representative chromosomal abnormalities from different metaphase cells

biologically intriguing neoplasm that highlights the gating strategies adopted in performing flow cytometric analysis. It also underscores the importance of a multi-disciplinary, integrated diagnostic approach towards these aggressive cancers.

Conflict of interest The authors declare that they have no conflict of interest.

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