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Case Report

A rare morphological and immunophenotypic presentation of adult B-cell acute lymphoblastic leukemia with blasts in the monocytic zone on CD45 side scatter

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ABSTRACT

We present a case of B-acute lymphoblastic leukemia in an elderly patient who presented with severe weakness and pancytopenia. The patient was a 75 year old Female whose blasts had an unusual morphology in form of coarse azurophilic granules and cytoplasmic blebs and on flow cytometry the blasts were present in the bright CD45 zone with a high side scatter. Bone marrow aspirate sample was subjected to multicolour flow cytometry using Beckman Coulter Navios® which is an 8 colour flow cytometer. Flow cytometric analysis of the bone marrow aspirate showed blasts in the monocytic zone with a precursor B cell immunophenotype. Complete blood counts showed pancytopenia with peripheral blood film not showing any blasts. Bone marrow aspirate smears showed 20% blasts with coarse azurophilic granules and cytoplasmic blebs. The position of the blasts in this case which were in monocytic zone giving them a bright expression of CD45 and a high side scatter on the CD45 side scatter. This is not the usual position for blasts in B- acute lymphoblastic leukemia as these blasts are less complex. A bright expression of CD45 by blasts in B- acute lymphoblastic leukemia is known to be associated with a poor prognosis but the clinical significance of blasts being bright CD45 with a high side scatter is a very rare occurrence and more number of cases with a similar presentation are required to determine a prognostic significance.

Keywords: B-acute lymphoblastic leukemia, Monocytic zone, Bright CD45 expression, High side scatter, Coarse azurophilic granules, Blebs

INTRODUCTION

B-acute lymphoblastic leukaemia is a neoplasm of precursor cells (lymphoblasts) confined to the B cell lineage. 80% of ALL occurs in children whereas in adults it presents as an aggressive disease. The incidence of ALL follows a bimodal distribution, with the first peak occurring in childhood and a second peak occurring around the age of 50.[1] Most patients of B-ALL present with evidence and consequences of bone marrow failure with the total leukocyte count being decreased, normal or markedly elevated. The blasts are morphologically variable with blasts ranging from homogenous small sized with a relatively condensed chromatin, inconspicuous nucleoli and a very scant amount of basophilic agranular cytoplasm to heterogenous blasts with a medium to large size, fine chromatin, 2 to 3 prominent nucleoli and a moderate amount of basophilic, agranular cytoplasm. On flow cytometry the blast in B-ALL usually have a low side scatter with a dim to negative expression of CD45 and a characteristically low side scatter.[2,3] The blasts in B-ALL are almost always positive for the B cell markers CD 19, cytoplasmic CD79a, CD10 and cytoplasmic CD22; with none of these by itself being specific. CD20 and CD34 expression is variable with not all cases being positive for these.[3,4]

Important immunohistochemical markers that are usually positive include PAX5 and TdT with the former considered to be very sensitive.[5] We present a 75 year old female patient who was diagnosed with B-ALL based exclusively on immunophenotyping. The patient presented with severe weakness and pancytopenia. The finding which was surprising was a high Side Scatter with a bright expression of CD45 on CD45 side scatter on flow cytometry with morphology showing coarse azurophilic granules with cytoplasmic blebs. This is a very rare occurrence for the blasts in B- acute lymphoblastic leukemia.

CASE REPORT

A 75 year old female patient was admitted to our hospital in October 2018 with severe weakness and pancytopenia. The bone marrow aspiration was done outside initially and was reported as acute myeloid leukemia with maturation (AML-M2). On admission the routine investigations were done which primarily included complete blood counts (CBC), peripheral blood film (PBF), a repeat bone marrow aspiration for morphological examination and flow cytometric immunophenotyping. CBC showed hemoglobin level of 8.8 g%, total leucocyte count of 1,100/µL, platelet count of 69,000/µL with a differential count showing 41 neutrophils, 53 lymphocytes, 4 monocytes and 2 eosinophils. Peripheral blood film did not reveal any blasts.

Bone marrow (BM) examination demonstrated a cellular marrow showing exactly 20% blasts with a normoblastic to megaloblastic erythropoiesis and increased iron stores. Myelopoiesis appeared suppressed with adequate megakaryocytes. There was a mild prominence of plasma cells (~06%). The blasts in this case had an unusual morphology with medium to large sized blasts with presence of coarse azurophilic granules with many showing cytoplasmic blebs. The special stain MPO, NSE and SBB were negative so we could just give a morphological diagnosis of acute leukemia. The diagnosis of B-ALL was confirmed by enumerating number of blasts on bone marrow aspirate smears along with a correlation with flow cytometric immunophenotyping. The patient could not be followed up further as she was lost on follow-up.

IMMUNOPHENOTYPING

The bone marrow sample was adjusted to a cell count of 5,00,000 cells/µL and 100 µL was taken in each tube. A total of 19 Antibodies were used in this case. 15 surface antibodies and 4 cytoplasmic antibodies. The antibodies that were used are depicted in Table 1. The antibodies were procured from Beckman Coulter. The sample was incubated with the antibodies for 20 minutes. Then lysing solution (500 µL) was added and again incubated for 10 minutes and then given 3 washings and sample acquired. For cytoplasmic staining, the samples were treated with permeabilizing agent (Intra-Prep 1, Intra-Prep 2) and cytoplasmic antibodies added and incubated. The sample again washed 2 to 3 times and acquired. We acquired 50,000 events per tube and the results were analyzed with the Kaluza® software (Beckman Coulter).

Table 1: Antibody combinations with associated fluorochromes.

Fluorochrome	FITC	PE	ECD	PC5.5	PC7	APC	APC-AF700	APC-AF750
Antibody	CD7 CD20 CD36 CD64 MPO	CD79a CD117	CD45	CD3 CD10 HLA-DR	CD13 CD19	CD4 CD33 CD34	CD22 CD11c	CD14

RESULTS

The blasts were present in the monocytic zone with a bright expression of CD45 and a high side scatter. The blasts were positive for CD19, CD20, CD22, CD34, HLA-DR with an aberrant expression of CD33. The monocytic markers that include CD4, CD11c, CD14, CD36 and CD64 were negative. The T cell markers CD3 and CD7 were negative. The Myeloid marker that included MPO, CD117 and CD13 were negative.

DISCUSSION

B-acute lymphoblastic leukemia is a neoplasm of precursor cells (lymphoblasts) confined to the B cell lineage. B- Acute lymphoblastic leukaemia (B-ALL) is primarily a disease of children with 75% of cases occuring in children under six years of age. Unlike acute myeloid leukemia which can be diagnosed based on morphology due to presence of Auer rods and MPO positivity, B-ALL is a diagnosis which needs flow cytometric confirmation.[6] The blasts in ALL are usually small in size as compared to myeloblasts but ALL can show heterogeneity with many cases presenting with larger size and prominent nucleoli just like myeloblasts. For this reason the FAB category of ALL-L1 is at times indistinguishable from AML-M0. Besides the small sized blasts can be seen in T-ALL or acute megakaryocytic leukemia (AML-M7) therefore indistinguishable morphologically.[7,8] Also one would argue that if the Blasts show PAS positivity, then the diagnosis goes in favour of ALL. But due to a poor sensitivity and not so reliable specificity, the PAS stain on cytochemistry cannot be considered for lineage assignment for ALL

unlike MPO or NSE, which are considered as lineage specific markers.[9-11] PAS positivity in erythroblasts is considered to be a feature of dyserythropoiesis and is associated with a poor prognosis in MDS.[12] Therefore for diagnosis of B-ALL, one has to rely on flow cytometric immunophenotyping for confirmation. The blasts in B-ALL are usually small to medium sized and as per FAB classification as classified as ALL-L1 where the blasts are homogenous, small sized having a relatively condensed chromatin, absent to inconspicuous nucleoli and a very thin rim of basophilic cytoplasm. In ALL-L2 the blasts are heterogenous with a large size, fine chromatin, 2 to 3 prominent nucleoli and a moderate amount of basophilic, agranular cytoplasm. This morphology is reminiscent of type 1 myeloblasts (myeloblasts are of 3 types; the type 1 is similar to ALL-L2 explained above, in type 2 myeloblasts there are few granules <20 and the type 3 myeloblasts are characterized by presence of extensive granulation >20). To differentiate the two morphologically is not possible especially when you have large agranular blasts with no Auer rods. Though in a subset of cases of B-ALL we can get coarse azurophilic granules but these granules are negative for MPO. The blasts in ALL usually have well defined cytoplasmic borders. Cytoplasmic blebs though not specific for megakaryoblasts are frequently seen in AML-M7. In our cases the blasts showed both coarse azurophilic granules in a few and cytoplasmic blebs in few of the blasts (Figure 1).[13]

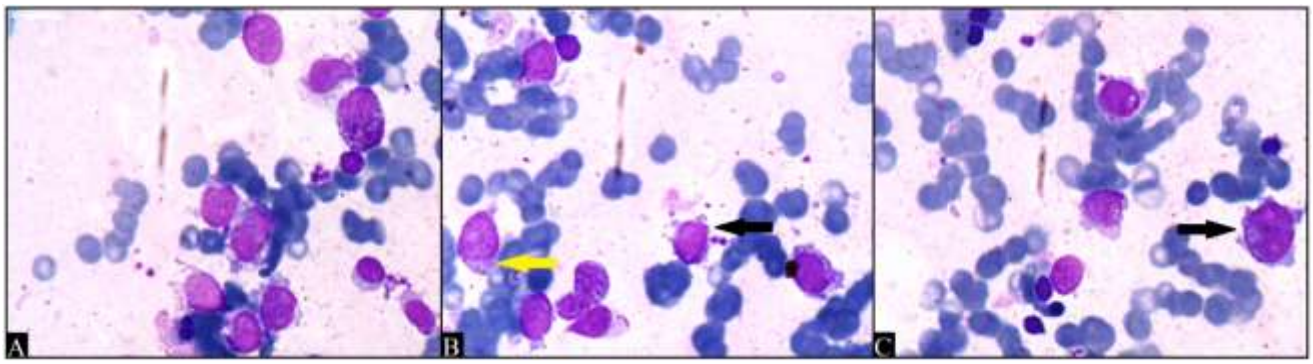

Figure 1: Bone marrow aspiration showing blasts.

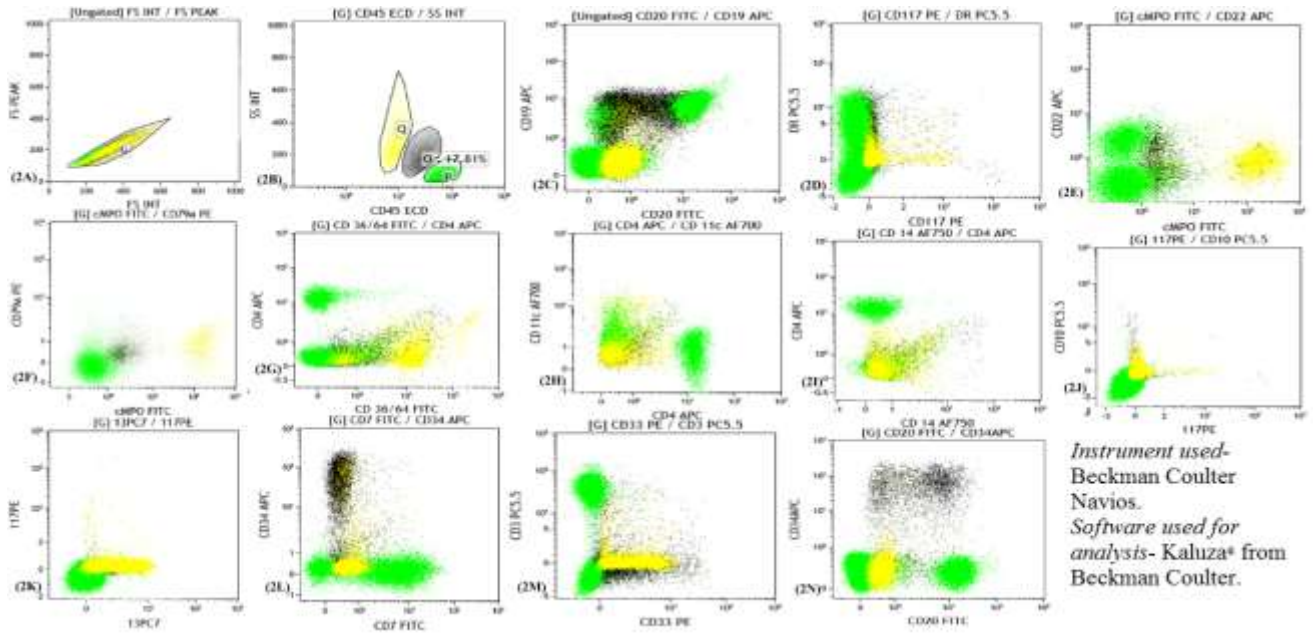

Figure 2: Immunophenotyping of B-ALL. (A) shows the singlet gate displayed on FS peak vs FS int; (B) shows CD45/SSc with blasts in the monocytic zone (region O). These cells have a bright CD45 expression with a high side scatter (~17.81%); (C) shows the blast population being positive for CD19 and CD20; (D) shows HLA-DR positivity with a negative expression of CD117; (E) shows CD22 positivity with a negative expression of MPO; (F-K) shows a negative expression of CD79a, CD4, CD36, CD64, CD11c, CD14, CD10 and CD13; (L) shows a bright expression of CD34 with a negative expression of CD7; (M) shows an aberrant expression of CD33 with a negative expression of CD3; (N) shows positivity for CD20 and CD34.

The blasts are medium to large sized showing a high nuclear cytoplasmic ratio and fine nuclear chromatin with blasts showing cytoplasmic blebbing (black arrow) and some of these blasts showing coarse azurophilic granules (yellow arrow). These blasts were scattered in this cellular marrow and constituted exactly 20% of all nucleated cells (Figure 1).

Therefore relying on flow cytometry is the best modality for an exact diagnosis. The blasts in B-ALL usually show a dim to negative CD45 expression unlike other acute leukemias which are usually dim but not CD45 negative. B-ALL blasts usually have low side scatter unlike AMLs wherein because of a more complex structure due to presence of granules have a higher side scatter. Also B-ALL usually have dim expression of CD45. A bright expression of CD 45 in B-ALL is reported and is usually associated with an aggressive outcome. In our case the blasts had both high side scatter and a bright expression of CD45, thus putting the blasts in the unusual monocytic zone. This was probably due to granulation and cytoplasmic blebs in these blasts which gave them a high side scatter. The confirmation of these blasts being B cell lymphoblasts was confirmed by positivity for CD19, CD20, CD22 along with CD34 and HLA-DR and an aberrant expression of a myeloid associated antigen CD33 (Figure 2).

CONCLUSION

Our case defied the normal presentation of B-ALL both on morphology as well as on flow cytometric analysis. The patients with a bright expression of CD45 is associated with poor prognosis and also the patients having granulated blasts in B-ALL is associated with a worse prognosis especially in children.[14] The prognostic relevance of lymphoblasts showing cytoplasmic blebs and a high side scatter could not be determined due to a very rare occurrence, though a high side scatter was reported to be associated with poor prognosis in DLBCL. More number of cases would be required for analysis to find the relevance of these parameters if at all there is any.

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