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CASE SERIES

Rare Absence of CD79alpha in B-ALLs is not Associated with Altered Prognosis

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ABSTRACT

Background: The diagnosis of B-cell acute lymphoblastic leukemia (B-ALL) on the basis of the World Health Organization (WHO) recommendations in 2016 requires the expression of CD19 with CD79a (CD79a), cCD22 and CD10. CD79a is an integral B cell antigen expressed at all stages. Its absence in B-ALL has only been rarely reported. In this study, we described three cases of B-ALL showing complete loss or extremely weak expression of CD79a.
Methods: Seventy-five patients with B-ALL diagnosed by flow cytometry over a period of 3.5 years were taken out from the records. Cases lacking for CD79a by flow cytometry were further analysed.
Results: Out of 75 cases, CD 79a negativity was seen in 4% (3/75). However, every three cases expressed other B cell markers. No aberrant expression of myeloid and lymphoid antigens was seen in any of these three cases. The prognosis of these three cases was similar to CD79a positive cases.
Conclusion: Rarely, B cell ALL may lack CD79a. Thus, to avoid the misdiagnosis, a comprehensive antibody panel should be used. There was no prognostic impact of CD79a negativity in this study, however larger studies are needed to confirm these results.

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Introduction

Current standards for diagnosis of acute lymphoblastic leukemia (ALL) include the study of cell morphology, immunophenotyping and cytogenetics as detailed in the 2016 WHO classification of lymphoid neoplasms. Immunophenotyping by means of multicolour flow cytometry (MFC) has become the gold standard procedure for diagnosis of ALL and its subclassifications. The most important markers used for diagnosis of B-lineage include CD19, CD10, CD22, and CD79a.[1]

CD79a is believed to be a B cell–specific antigen as its expression begins at the earliest pro-B stage of development, preceding the expression of CD19 and continues throughout B cell differentiation to the late plasma cell stage.[2] This makes it one of the most useful and diagnostically sensitive pan–B-cell markers for immunophenotypic analysis. While its presence in combination with CD19, CD10 and CD22 is considered diagnostic for B-ALL, its absence in a case of B-ALL has been only rarely reported. Here, we report three cases of B-ALL showing complete loss or extremely weak expression of CD79a.

Materials and Methods

This is a retrospective study carried out in hematology section of Department of Pathology of a tertiary care hospital in Delhi, over a period of 3.5 years. Seventy-five cases diagnosed as B-ALL over these years were evaluated for immunophenotypic expression of CD79a. Complete clinical history, physical examination and investigations of these cases were noted from the records.

Complete blood cell count was done on Beckman coulter LH 500. Peripheral blood and bone marrow aspirate smears were stained with Wright's stain and cytochemical staining was performed for myeloperoxidase (MPO) activity of the blasts.

Immunophenotyping of the blasts were performed on lysed whole peripheral blood or bone marrow aspirates using flow cytometer (Beckman coulter cytomics FC 500) equipped with facility for at least 5-color flow cytometry.

The following panel was used for characterisation of the blasts: Immaturity markers: CD45/CD34/TdT/HLADR, myeloid markers: cMPO/CD117/CD13/CD33/CD64, T lymphoid markers: cCD3C/D3/CD5/CD7 and B-lymphoid markers: CD79a/CD10/CD19/CD20.

A threshold of 20% was used to define a positive reaction of the blast cells to a given monoclonal antibody, except for cMPO, cCD3, cCD79a and TdT, which were considered positive at 10% level of expression.[1] Patients were diagnosed as B-ALL on the basis of WHO criteria i.e. strong CD19 expression plus strong expression of at least one of CD79a, cCD22, CD10 or weak CD19 expression plus strong expression of at least two of CD79a, cCD22, CD10 markers.[3]

Minimal residual disease (MRD): Cell preparation was done using stain-lyse-wash method on bone marrow aspirate sample using flow cytometer (Beckman coulter cytomics FC 500) equipped with facility for at least 5-color flow panel. The following markers were used: CD45, CD10, CD19, CD20, CD34, CD38, CD58, CD128, CD123, CD86 and CD200. A cut-off of 0.01% was used to define MRD positivity. The statistical analysis was done using FCS express software version 3.0.

Results

Out of 75 cases of B-ALL, 71/75 were CD10 positive (Pro-B-ALL). CD 79a was negative in 4% (3/75) of cases. Out of 71 CD10 positive cases, 3/71, 4.2% were negative for CD79a while all 4 CD10 negative cases were CD79a positive. Detailed clinical presentation, laboratory work up and outcome of these three cases are summarized in Table 1. All three cases had thrombocytopenia with anemia of varying severity. No aberrant expression with respect to any marker was noted in any of the three cases. Cytogenetic analysis was performed in three cases with CD79a negativity which were negative for t (9,22) by FISH. They were treated by standard BFM-95 protocol and responded well to the treatment. Cytopenias improved with simultaneous resolution of clinical symptoms and resolution of organomegaly. MRD was negative at the end of the induction period as well as at the end of maintenance period (followed for 1 year) in two cases. The third case was lost to follow up after induction. Figure 1 shows the immunophenotyping of the blasts from one of these cases.

Discussion

The diagnosis and classification of acute leukemia is currently a multistep procedure based on morphology, immunophenotyping, cytogenetics and molecular genetics. MFC is extensively used in the diagnosis of almost all hematologic malignancies because of its capacity to analyze multiple markers simultaneously on the same cells.

A strong expression of CD19 plus strong expression of at least one of CD79a, cCD22, CD10 or weak expression of CD19 plus strong expression of at least two of CD79a, cCD22,

Table 1: Clinical, and Laboratory Features of B-ALL patients with negative expression of CD79a

	Case 1	Case 2	Case 3
Age (Years)/ Gender	6/ Male	19/ Female	10/ Male
Symptoms	Fever, abdominal pain	Shortness of breath, rectal bleeding	Gum bleeding
Physical examination	Splenomegaly, cervical lymphadenopathy	Splenomegaly	Splenomegaly
CBC			
Hemoglobin	4.7 g/dL	6.0 g/dL	9.0 g/dL
Leucocyte Count	130 $\times 10^9$/L	13.7 $\times 10^9$/L	18 $\times 10^9$/L
Platelets	25 $\times 10^9$/L	9.0 $\times 10^9$/L	66.0 $\times 10^9$/L
Bone Marrow			
% of Blasts	76%	44%	42%
Cytochemical MPO	Negative	Negative	Negative
FAB Type	L1	L1	L2
Immunophenotyping			
CD45	Dim	Negative	Dim
CD10	Bright	Bright	Moderate -Bright
CD19	Moderate	Moderate	Moderate
CD20	Heterogenous	Heterogeneous	Heterogeneous
HLA-DR	Moderate	Moderate	Moderate
TdT	Dim	Dim	Dim
CD34	Moderate	Moderate	Bright
cCD79a	Negative	Negative	Negative
cMPO	Negative	Negative	Negative
cCD3	Negative	Negative	Negative
Aberrant expression of immunophenotypic markers	None	None	None
Cytogenetic analysis by (FISH)	Negative	Negative	Negative
MRD analysis	Negative (post induction and maintenance)	Negative (post induction and maintenance)	Negative (post induction)

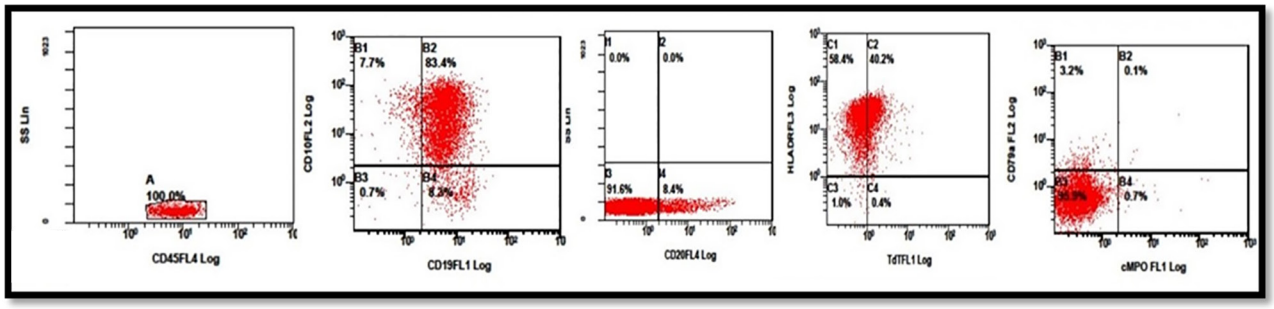

Figure 1: Blasts with dim expression of CD45, positive for CD19, CD10, HLA DR and TdT, heterogeneous CD20, and negative for CD79a and cMPO.

CD10 markers is diagnostic of B-ALL.[3] However, these markers are expressed at variable stages of B cell development and hence exhibit variable sensitivity in diagnosing B-ALL.

CD10 is a common ALL antigen (CALLA) and is expressed on both B-ALL as well as T-ALL, however it is negative in cases of pro-B ALL[3-6] CD20 is a glycosylated phosphoprotein expressed on the surface of all B-cells (except early pro-B cells and plasma cells). It is expressed only in approximately 50–60% of B-ALLs.[7, 8] CD19 is considered as a Pan B-cell marker as it is expressed on B-cells from earliest recognizable B-lineage cells during development, i.e. B-cell blasts (hematogones) to the terminal differentiation stage of B-cells, i.e. plasma cells.[3] Hence, it has been extensively used in the diagnosis and characterization of B-cell malignancies.

CD79 is a dimeric transmembrane protein that is associated with membrane immunoglobulin and initiates B-cell signalling following engagement of the latter by antigen. The two component chains of CD79, referred to as CD79a and CD79b, are encoded by mb-1 and B29 genes, respectively.[9] Its expression begins at the earliest stage of development of B-cells (Pro-B cells), preceding the expression of CD19 and continues throughout the B-cell differentiation to the late plasma cell stage.[10-12] This makes it one of the most useful (ie, diagnostically sensitive) pan–B-cell markers being used in routine immunophenotypic analysis.

In the present study, three cases (4%) were negative for CD79a expression. Absence of CD79a is rarely reported in B-ALL. In a study conducted by Gujral et al, 1120 cases of B-ALL were analysed. Overall, 0.62% (7/1120) were negative for CD79a. Out of these, 1071/1120 were CD10 positive, while 49/1120 were CD10 negative. All CD10 positive cases showed CD79a antigen expression, while 22% (7/32) of pro B-ALL (CD10 negative) cases were CD79a negative. The authors also found that expression of cCD22 (97%) to be more sensitive than CD79a (78%) as a lineage specific marker for pro B-ALL.[7] Out of 71 CD10 positive cases, 4.2% were negative for CD79a, while all 4 CD10 negative cases were CD79a positive. There was no case with negativity for both CD10 and CD79a markers in this study.

CD22 is a B lineage specific marker expressed in the cytoplasm of pro-B and pre-B cells and on the surface of more mature B cells. It is commonly used in the diagnostic panel for B-ALL.[13] Nasr MR et al. studied expression of CD22, CD79a and PAX-5 by immunohistochemistry in 34 B-ALL cases and found PAX5 to be a more sensitive marker than CD79a and CD22 for lineage assignment in B-cell ALL.[14] Similar findings were reported by Adams H and colleagues who studied 20 B-ALL patients and found that PAX5 and CD19 are more sensitive markers for B-lineage derivation than CD79a.[15]

No aberrant expression with respect to any markers was noted in any of the three cases with CD79a negativity in the present study. Cytogenetic analysis was performed in all the cases that were negative for t(9,22), t(4;11) and t(12;21). These cases were MRD negative at the end of induction, and two at the end of maintenance period. In addition, all three CD79a negative cases were CD10 positive which is known to be a good prognostic marker in B-ALL.[16] This is in contrast to the findings of Gujral et al. who found 7 cases of CD79a negative B-ALL who were also CD10 negative, a poor prognostic marker.[7]

Conclusion

Very few studies have reported absence of CD79a in B-ALL in literature. An absence or down regulation of CD79a can make the diagnosis of B-ALL challenging, especially in laboratories using acute leukemia screening panel with limited markers leading to incorrect diagnosis. Although rare, B-ALL can show negative expression of CD79a. Thus, to avoid the misdiagnosis of B-ALL, a comprehensive antibody panel including antibodies against other B-cell markers such as CD20, CD10, CD19, and cCD22 should be used. Immunohistochemistry for PAX-5 can also serve as a sensitive marker in such cases. It is suggested that larger studies are required to find out the prognostic impact of CD79a negativity in B-ALL.

Conflict of Interest: None declared.

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