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ABERRANT EXPRESSION OF CD MARKERS IN ACUTE LYMPHOBLASTIC LEUKEMIA: A DIAGNOSTIC CLUE OF MALIGNANCY OR COMPOUNDING CONFUSIONS

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ABSTRACT

Flow cytometric immunophenotyping currently comprises of standard of care test for acute lymphoblastic leukemia (ALL). Aberrant expression of CD markers is seen in several cases of acute leukemia due to their abnormal genetic programme. In present study we evaluated the frequency of aberrant expression of CD markers in 36 new cases of ALL patients using CD45/SSC gating. Out of these, 77.8 % had conventional immunophenotypes while 22.2% cases showed expression of CD antigens which were not of that specific lineage. The presence of aberrancy helps to identify a neoplastic process. To conclude aberrant immunophenotypes are quiet common in ALL and have important implications for the diagnosis, monitoring and also establish baselines for subsequent detection of MRD.

KEYWORDS : Acute leukemia, Flow cytometry, CD markers, Aberrant expression

Acute leukemia comprises a heterogenous group of malignancies with variable clinical, morphologic, and immunophenotypic features. (Momani et al., 2016) Immunophenotypic analysis is an essential component of the diagnostic work-up of acute leukemias (AL), and flow cytometry (FC) is the preferred method of analysis. (Hussein et al., 2011)

Acute lymphoblastic leukemia (ALL) is a hematological malignancy characterized by accumulation of uncontrolled proliferation of immature lymphoblasts in the bone marrow, peripheral blood, and, occasionally, the central nervous system. (Sharma et al., 2014) Two subsets of ALL are precursor B-cell ALL and precursor T-cell ALL (Suggs et al., 2007) based on the expression of various B-cell and T-cell lineage associated antigens.

Aberrant phenotypes in acute leukemia are defined as patterns of antigen expression on neoplastic cells different from the process of normal hematopoietic maturation.(Sharma et al., 2016) due to their abnormal genetic programme. These aberrancies include abnormal or asynchronous expression of normal hematogone associated antigens (where early antigens are co- expressed with mature ones) and Cross-lineage antigen expression (expression of myeloid antigens in ALL, B-lineage antigens in T-ALL or T-lineage antigens in B-ALL) or inappropriate expression of myeloid, T-lymphoid, and NK cellassociated antigens. (Momani et al., 2016) (Hussein et al., 2011) (Sharma et al., 2016).

From a prognostic point of view, aberrant antigen expression can adversely influence the clinical response, remission rate and overall survival in patients with acute leukemia (Momani et al., 2016). Flow cytometric immunophenotyping currently comprises of standard of care test for acute lymphoblastic leukemia (ALL). It helps to confirm the diagnosis, exclude neoplastic and non neoplastic mimics. In present study we evaluated the frequency of aberrant expression of CD markers in ALL.

MATERIALS AND METHODS

This study was conducted in the Department of Pathology of a tertiary care center in North India during the period of July 2015 to June 2016. During this period 36 new cases of ALL were diagnosed. Diagnosis of acute leukemia was based on morphological examination of bone marrow aspirate smears, including cytochemistry along with flow cytometric immunophenotyping. All the cases of ALL (B-ALL and T-ALL) were included in the study.

Peripheral blood/ Bone marrow aspirate samples were processed using a standard stain-lyse-wash method for flow cytometric immunophenotyping. The samples were processed within 24 h of collection. The monoclonal antibodies used were: CD45(APC-H7), CD13(PE-Cy7), CD33 (APC), CD10 (PE), CD19 (PE-Cy7), CD7 (APC), CD117 (PE), CD34 (PerCP-Cy5.5), HLA-DR (PerCP-Cy5.5), MPO (FITC), cCD79a (PE), cCD3 (PE-Cy7), Tdt (APC), CD64 (FITC), CD 20 (APC), CD8(FITC), CD4 (PE) and CD5(PerCP-Cy5.5). All of these antibodies were purchased from BD Biosciences (San Jose, CA, USA).

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Initial incubation of monoclonal antibodies for 15-30 min at room temperature in the dark followed by lysis and washing of debris/unlysed RBCs were performed and samples were acquired using a 8 Color Flowcytometer BD FACS Canto II (Becton Dickinson, San Jose,CA) system. All analyses and interpretation were carried out using the FACS-Diva software (BD Biosciences). Aberrant antigen expression was defined as ≥20% of blasts showing one or more of CD13, CD33, and CD117 antigens.

Results were obtained by gating the blast cells with side scatter (SSC) versus forward scatter (FSC) followed by SSC versus CD45 gating. Doublets were excluded based on 'area versus width' or 'area versus height' plotting. FCM data was analyzed based on dot plots. For surface antigens, marker positivity was considered when more than 20% of blast cells were positive and more than 10% blast cell positivity was considered for cytoplasmic antigens.

RESULTS

In the present study, 36 patients of ALL were analyzed to find out the frequency of aberrant antigens expression. There was slight male predominance with male to female ratio of 1.6:1. Average age of the patients was 24.2 years (range 2-70 years). Physical examination revealed hepatomegaly in 69% of the cases, splenomegaly in 56.5% and lymphadenopathy (LAP) in 62.3% of the cases. Hematological parameters showed mean Hb 6.8 gm% (3.1-11.3 gm%), mean TLC 63 × 10^9/L (2 × 10^9/L- 380 × 10^9/L) and mean platelet count 34 × 10^9/L (2 × 10^9/L -185 × 10^9/L) .

These 36 cases of ALL were further subdivided into B-ALL and T-ALL. On analysis of immunophenotypic expression, 32 (88.9%) patients had B-ALL and 4 (11.1%) patients had T-ALL. Lineage specific Lymphoid and myeloid cell markers in various stages of maturation have distinct immunophenotype. (Table 1)

Out of these 36 cases, 28 (77.8 %) had conventional immunophenotypes as they showed expression of lineage specific markers while 8 (22.2%) cases showed expression of CD antigens which were not of that specific lineage. Seven cases (21.8%) of B-ALL and one (25 %) case of T-ALL expressed aberrant markers. (Table 2)

In B-ALL, most common aberrant marker was CD13 present in 5 cases (15.6%), followed by CD33 in 2 cases (6.2%). One case was positive for CD7 (3.1%). One case co- expressed CD13 and CD33. Infidelity marker seen in one case of T-ALL was CD79a (25%). (Table 3) (Figure 1,2)

DISCUSSION

The majority of acute leukemia cases express specific lineage markers; however, there is a variable number of cases in which aberrant immunophenotypes can be detected. The frequency of aberrant expression of CD markers in our study was 22% similar to other studies done by Weirsma et al., 1991, Bhushan et al., 2010 and Shen et al., 2003. with 22%, 23% and 27% respectively.

We assessed the relationship between aberrant myeloid marker associations with other variables at the time of diagnosis of ALL. Age, gender, morphology of blast in peripheral blood, leucocyte count and hemoglobin were not significant factors in this study which was similar to study done by Kurec et al., 1991.

However; in our study CD34 expression was significantly associated with aberrant expression of myeloid markers in B-ALL. CD34 is normally expressed on hematopoietic stem cells and early thymic T-cell precursors. Expression of CD34 on leukemic blasts might be associated with developmental arrest at an immature stage or continuous spectrum of expression. The results are in agreement with previous study by Vitale et al., (2007), where CD34 expression was significantly associated with myeloid associated markers.

Most common immunophenotypic aberrancy was expression of myeloid associated antigen seen in 6 cases (18.7%) of B-ALL. WHO 2008 (Borowitz, 2008) also states that myeloid markers CD13 and CD33 are often expressed in ALL cases. Our study upheld the WHO suggestion in relation to CD13 and CD33 where expression of these markers was found 15.6% and 6.2% respectively. Similar to earlier reports CD13 was the most frequently expressed antigen in our study (Vitale et al., 2007) (Seegmiller et al., 2009). One case of B-ALL was positive to CD7, a T-cell marker, similar to the study done by lopes et al., 2014.

In our study, one case of T-ALL showed aberrant expression of CD79a which is a B-cell marker. This is similar to that

Table 1 : Lineage specific markers

Myeloid Markers	B- Cell Markers	T- Cell Markers
CD13	CD19	CD3
CD33	CD20	CD7
CD117	CD10	CD4
CD64	CD79a	CD8
MPO	Tdt	CD5

Table 2: Pattern of CD markers expression in ALL

	Number of Case	Conventional Cases	Aberrant Cases
B- ALL	32 (88.9%)	25 (78.1%)	7 (21.8%)
T- ALL	4 (11.1%)	3 (75%)	1 (25%)

Table 3: Distribution of aberrant CD markers in ALL

CD Markers	B- CELL AL (32/36)	T- Cell ALL (4/36)
CD13	5 (15.6%)	-
CD33	2(6.2%)	-
CD7	1(3.1%)	-
CD79a		1 (25%)

published by WHO 2008 (Borowitz MJ ; 2008) classification which stated that CD79a may be observed in 10% of T-ALL cases.

Correlation with treatment response had not been carried out so definite comment upon overall prognostic significance of these aberrant markers cannot make in our study. However; presence of aberrancy helps in identifying a neoplastic process.

Some limitation of our study is small number of cases and correlation of the presence of aberrant markers with treatment response, overall survival and prognosis was not done. Therefore, large scale study need to be conducted which should include all these important variables along with cytogenetics because some aberrant antigen expression may be associated with a particular karyotypic abnormality.

Figure 1 : B-All with aberrant expression of CD7

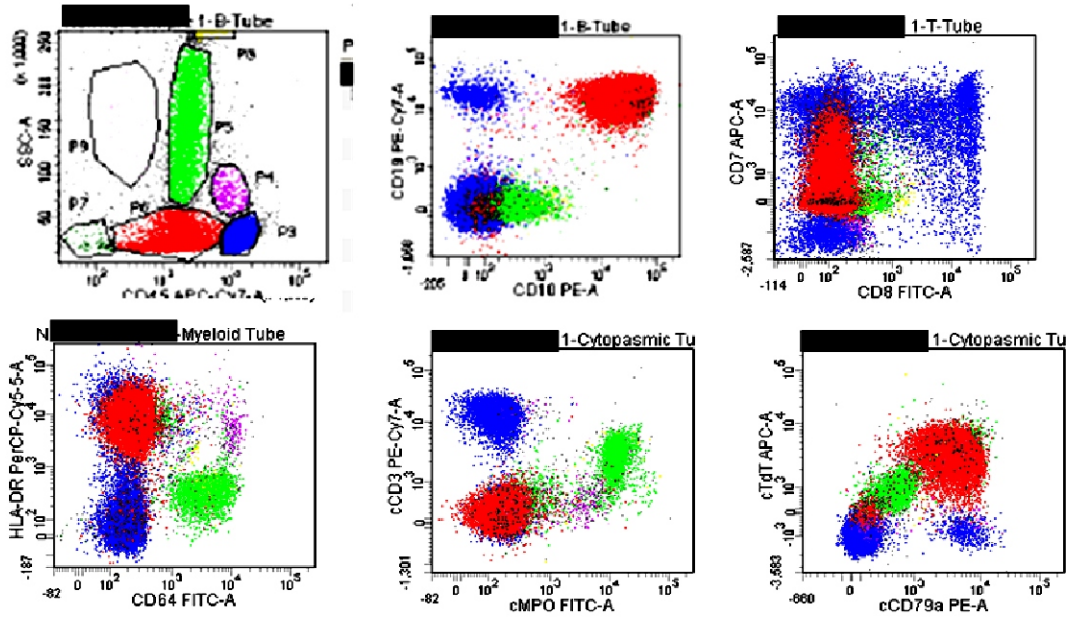

Figure 1: Dot plot SSC/CD45 gating showing dim CD45, coexpression of CD10 & CD19, positive for HLA-DR , CD79a, Tdt and aberrant expression of CD7 and negative for MPO and cCD3.

Figure 2 : B-All with aberrant expression of CD13 & CD33

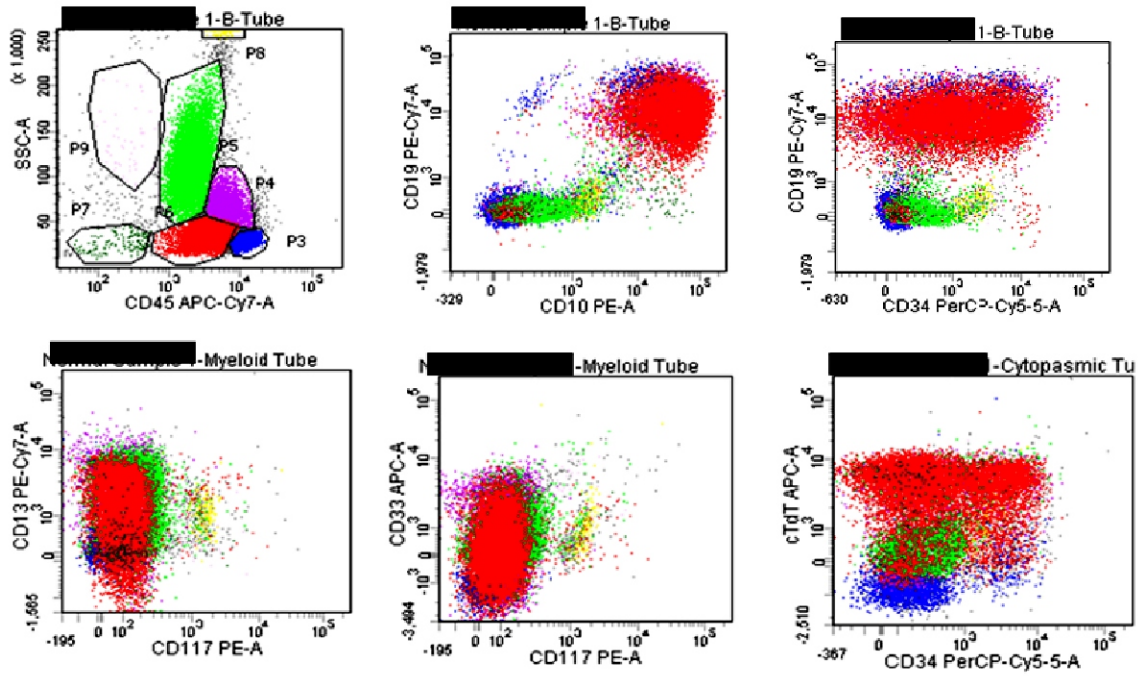

Figure 2: Dot plot SSC/CD45 gating showing dim CD45, coexpression of CD10 & CD19, positive for CD34, CD79a, Tdt, aberrant expression of CD13 and CD33 and negative for CD117.

CONCLUSION

The incidence of aberrant antigen expression in ALL cases was comparable with most of the published data. It has been demonstrated that several immunophenotypes of blast cells from cases of ALL do not exhibit the features of normal cellular differentiation but show the expression of aberrant markers.

To conclude, aberrant immunophenotypes have important implications for the diagnosis, monitoring and also subsequent detection of MRD in ALL.

REFERENCES

Bhushan B, Chauhan PS, Saluja S, Verma S, et al. , 2010. Aberrant phenotypes in childhood and adult acute leukemia and its association with adverse prognostic factors and clinical outcome. Clin. Exp. Med, **10**: 33-40.

Borowitz MJ. Acute leukemias of ambiguous lineage. In: Swerdlow, SH, Campo E, Harris NL, editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon: IARC Press., 2008: 150- 155.

Hussein S, Gill KZ, Sireci AN, Colovai AI, Small T, Emmons FN, et al., 2011. Aberrant T-cell antigen expression in B lymphoblastic leukaemia. British Journal of Haematology,**155**: 449-56.

Kurec AS, Belair P, Stefanu C, Barrett DM, et al. ,1991. Significance of aberrant immunophenotypes in childhood acute lymphoid leukemia. Cancer, **67**: 3081-6.

Lopes T, Andrade K, Camelo N, Rodrigues V, Oliveira R., 2014. Influence of aberrant myeloid expression on acute lymphoblastic leukemia in children and adolescents from Maranhão, Brazil. Genetics and Molecular Research ,**13**(4):10301-7.

Momani A, Abbasi N, Alsokhni H, Habahbeh L, Khasawneh R, Kamal N., 2016. Aberrant Antigen Expression in Patients with Acute Leukemias, Experience of King Hussein Medical Center in Jordan.JRMS, 23(2):59-67/DOI:10.12816/0027107

Sharma RK, Purohit A, Somasundaram V, Mishra PC, Kotru M, Ranjan R , et al., 2014. Aberrant myeloid antigen co-expression is correlated with high percentages of CD34-positive cells among blasts of acute lymphoblastic leukemia patients: an Indian tertiary care center. Blood Res**.**, **49**:241-5.

Suggs JL, Cruse JM, Lewis RE., 2007. Aberrant myeloid marker expression in precursor B-cell and T-cell leukemias. Experimental and Molecular Pathology, **83**: 4713.

Sharma M, Sachdeva MUS, Varma N, Varma S, Marwaha RK., 2016. Characterization of immunophenotypic aberrancies in adult and childhood acute lymphoblastic leukemia: A study from Northern India. Journal of Cancer Research and Therapeutics , **12** (2): 620-6.

Shen HQ, Tang YM, Yang SL, Qian BQ, et al. ,2003. Immunophenotyping of 222 children with acute leukemia by multi-color flow cytometry. Zhonghua Er. Ke Za Zhi ,41: 334-7.

Seegmiller AC, Kroft SH, Karandikar NJ, McKenna RW., 2009. Characterization of immunophenotypic aberrancies in 200 Cases of B acute lymphoblastic leukemia. Am J Clin Pathol,**132**:940-9.

Vitale A, Guarini A, Ariola C, Meloni G, Perbellini O, Pizzuti M, 2007. Absence of prognostic impact of CD13 and/or CD33 antigen expression in adult acute lymphoblastic leukemia: Results of the GIMEMA ALL 0496 trial. Haematologica, **92**:342-8.

Wiersma SR, Ortega J, Sobel E and Weinberg KI, 1991. Clinical importance of myeloid-antigen expression in acute lymphoblastic leukemia of childhood. N. Engl. J. Med, **324**: 800-08.