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ACUTE MYELOID LEUKEMIA: CORRELATION OF IMMUNOPHENOTYPING WITH MORPHOLOGY

Pathology

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ABSTRACT

Immunophenotyping by flowcytometry is fundamental to diagnose and subtype Acute Myeloid Leukemia. The objective of study to investigate immunophenotypes of different morphological FAB subtypes of AML. All newly diagnosed cases of AML(180 cases) on morphology that is presented to our hospital from January 2015 to December 2015 were included in study. Full panel of flowcytometric immunophenotyping was performed on BM and peripheral blood samples. Expression of myeloid markers and aberrant markers are studied. The results highlighted the correlation of a morphological FAB subtypes with immunophenotyping markers

KEYWORDS

Acute Myeloid Leukemia, Immunophenotyping, Flow cytometry

INTRODUCTION:

Acute leukemias are heterogeneous group of malignancies[1]. Acute myeloid leukemia (AML) is 20% of acute leukemia in children and 80% acute leukemia in adults.[2] Immunophenotyping(IPT) is fundamental to diagnose and subtype AML. In recent years, along with the wide application of AML immunophenotype testing, its relationship with genetics and morphology became better understood [3,4]

AIM AND OBJECTIVE: To investigate IPT markers of different morphological FAB subtypes of AML.

MATERIAL AND METHODS : The study was conducted in the Department of Pathology, Gujarat Cancer and Research Institute, Ahmedabad, Gujarat, India.

INCLUSION CRITERIA: All morphologically diagnosed cases of acute myeloid leukemia(AML) from January 2015 to December 2015.

EXCLUSION CRITERIA: All morphologically diagnosed cases of acute lymphoblastic leukemia (ALL).

Sample collection and preparation:

The bone marrow or peripheral blood was collected in ethylenediaminetetraacetic acid (EDTA) vacutainer for peripheral smear examination and immunophenotyping. A morphological evaluation was done from the Wright-stained peripheral smears and bone marrow aspirates using French–American–British classification of acute myeloid leukemias. Special relevant cytochemical stains like PAS and Sudan black were performed on the bone marrow aspirates in all cases. All the samples were processed within 24 hours.

Multicolor monoclonal antibody combination

The monoclonal antibodies used in the primary panel surface markers were CD45 (PerCP), CD22 (FITC), CD34 (PE), CD5 (PE Cy7), CD10 (APC), CD19 (APC-H7), CD7 (FITC), CD13 (PE), CD33 (PE Cy7), CD117 (APC), HLA-DR (APC-H7).The monoclonal antibodies used in primary panel cytoplasmic markers were MPO (FITC), cCD79a (PE), cCD3 (PE Cy7), and Tdt (APC). The monoclonal antibodies used in secondary panel were CD11b (PEcy7), CD11c(PE), CD14(APC-H7), CD15(FITC), CD2(FITC), CD4(PEcy7), CD8 (APCH7), CD1a (PE), CD41b(FITC), CD61 (FITC). The CD45 was used for blast gating for both surface and cytoplasmic markers. The antibodies were procured from BD Biosciences, USA.

Flow cytometric immunophenotyping

For surface markers, respective antibody (20 µl) mentioned above was added in six-color combination to the bone marrow or peripheral blood (100 µl) and incubated for 15 min. After incubation, 2 ml of erythrocyte lysing solution (1:10 dilution with double distilled water; BD Biosciences, USA) was added and incubated for 15 min at room temperature. Then, cells were centrifuged at 400 g for 5 min and supernatant was discarded. Remaining pellet was washed twice with phosphate-buffered solutions (PBS) and then suspended in 500µl of PBS. For cytoplasmic markers, 2 ml lysing solution was added to 100 µl of bone marrow or peripheral blood to lyse red blood cells and incubated for 15 min. After centrifugation to the pellet 1 ml perm/wash buffer was added to permeabilize the cells for intracellular staining and incubated for 20 min. After centrifugation to the pellet respective antibody (20µl) was added to the pellet and incubated for 15 min. Then, 2 ml PBS was added and the samples were centrifuged at 1500 rpm for 5 min. The supernatant was discarded and the pellet was suspended in 500 µl of PBS. For surface and cytoplasmic markers, negative control tubes were run simultaneously with the addition of sample and CD45 antibody.

Acquisition and data analysis

The cytometer setup and tracking beads were (BD Biosciences, USA) used for daily calibration of the instrument. The samples were then acquired in FACSCanto II flow cytometer (6-color, 2-Laser, BD Biosciences, USA) and analyzed using FACSDiva software (BD Biosciences, USA). At least 30,000 total cells were acquired, and the side scatter versus CD45 PerCP dot plot was used for blasts gating. The percentage of positive cells more than 20% was considered positive for that surface or intracellular markers. For MPO, cCD79a and cCD3 – more than 10% should be considered as positive.

Statistics: The SPSS software, 2017 version and Microsoft excel, 2007.

RESULTS: Total 180 cases were included from January 2015 to December 2015 in this study.

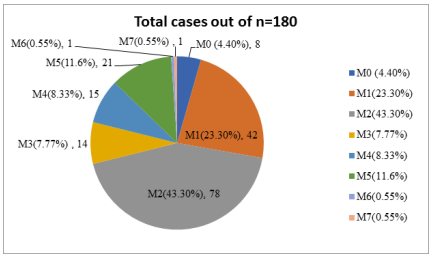

Figure 1: Percentage of morphologically diagnosed cases of Acute Myeloid Leukemia:

So, maximum number of cases were of M2 subtype (43.30%) and least number of cases were of M6(0.05%) and M7(0.05%) subtype.

Table 1: Expression of primary panel surface IPT markers with morphological FAB subtypes

Primary panel surface markers	M0 n=8	M1 n=42	M2 n=78	M3 n=14	M4 n=15	M5 n=21	M6 n=1	M7 n=1	%Positive rate n=180
CD34	7	28	59	1	9	8	1	1	114 (63.3%)
CD13	7	38	73	14	15	19	1	1	168 (93.3%)
CD33	8	39	71	14	15	21	1	1	170 (94.4%)
CD117	6	35	72	8	8	11	1	1	142 (78.8%)
HLA-DR	7	31	73	5	15	20	1	1	153 (85.0%)
CD5	1	1	0	1	0	0	0	0	3(1.6%)
CD10	0	1	0	1	0	1	0	0	3(1.6%)
CD19	0	1	5	4	1	1	0	0	12 (6.6%)
CD22	3	1	4	0	1	1	0	0	10 (5.55%)
CD7	2	4	11	2	2	3	0	0	24 (13.3%)

In primary panel surface markers, CD13 (93.3%) CD33 (94.4%) are expressed in maximum number of cases out of 180 cases.
In primary panel surface markers, CD5 (1.6%) and CD10 (1.6%) are expressed in minimum number of cases out of 180 cases.
In primary panel surface markers, CD7 (13.3%) is the most expressed aberrant marker. CD7 is expressed in maximum number of M0 cases (25%).

Table 2: Expression of primary panel cytoplasmic IPT markers with mophological FAB subtypes

Primary panel cytoplasmic markers	M0 n=8	M1 n=42	M2 n=78	M3 n=14	M4 n=15	M5 n=21	M6 n=1	M7 n=1	% Positive rate n=180
MPO	4	29	63	14	10	9	1	1	131 (72.2%)
cCD79a	2	3	2	0	0	1	0	0	8 (3.8%)
cCD3	0	0	0	0	0	0	0	0	0%
Tdt	8	3	7	0	1	0	0	0	19(10.5%)

Tdt is expressed by 100% cases of M0 subtype.
Total 10.5 % cases have expressed Tdt as a aberrant marker.

Table 3: Expression of secondary panel IPT markers with mophological FAB subtypes

Secondary panel markers	M0 n=8	M1 n=42	M2 n=78	M3 n=14	M4 n=15	M5 n=21	M6 n=1	M7 n=1	% Positive rate n=180
CD11b	1	2	10	0	14	21	0	0	47 (26.6%)
CD11c	2	10	12	0	15	21	0	0	60 (33.3%)
CD14	1	5	5	0	15	21	0	0	47 (26.1%)
CD15	3	6	7	0	14	21	0	0	51 (28.3%)
CD2	0	0	0	0	0	0	0	0	0%
CD4	0	0	0	0	0	0	0	0	0%
CD1a	0	0	0	0	0	0	0	0	0%
CD41	0	0	0	0	0	0	0	1	1(0.5%)
CD61	0	0	0	0	0	0	0	1	1(0.5%)

In secondary panel markers only M4 and M5 cases express monocytoid markers CD11b, CD11c, CD14 and CD15.

CD41 and CD61 is only expressed by M7 subtype of AML.
Secondary panel lymphoid markers are not expressed in any of the AML subtypes.

DISCUSSION:

All CD45 gated blasts of AML have expressed mainly CD13,CD33, CD117, MPO in 180 cases.

Table 4: Comparison of myeloid markers in AML cases with other studies

IPT myeloid marker	Present study 2017	Paietta et al [5] 2003	Zheng J et al[9] 2008
CD13	93.3%	95%	87%
CD33	94.4%	96%	96%
CD117	78.8%	80.6%	81.7%
HLA-DR	85.0%	85%	72.8%

So, expression of myeloid markers in present study is almost comparable with other studies.

Table 5 : Comparison of percentage of aberrant markers in AML cases with different studies

IPT marker	Present study 2017	Kaleem Z et al[6] 2003	Auewarakul CU et al [7] 2009	Khalidi HS et al [8] 2003
CD5	1.6%	15.9%	16.29%	17.22%
CD10	1.6%	16.12%	16.33%	18.45%
CD19	6.66%	16.66%	19.10%	17.55%
CD22	5.55%	18.1%	18.77%	18.10%
CD7	13.3%	17.20%	16.20%	16.20%

Kaleem Z et al[6], Auewarakul CU et al [7] and Khalidi HS et al[8] indicated that the positive rates of CD5,CD10,CD19,CD22 ,CD7 were between 16 and 20% in their study which is showing geographical differences between present(Indian) and other(European) population. Present study (Indian population) have lower percentage of aberrant markers.

CONCLUSION:

There are specific immunophenotyping markers for each FAB subtype of AML. Aberrant markers are also expressed by AML. CD7 and Tdt are the two most commonly expressed aberrant markers. There are specific cytogenetics for each subtype of AML. Immunophenotyping provides base for further molecular work up of AML.

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