Flow cytometry a fundamental tool for the diagnosis and subclassification of acute myeloid leukaemia: A retrospective study of immunologic profile of 198 newly diagnosed cases of acute myeloid leukaemia

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ABSTRACT

INTRODUCTION: Immunophenotyping by flow cytometry offers a rapid, precise and effective means for the diagnosis of acute myeloid leukaemia (AML). It provides valuable information of myeloid lineage assignment of the leukaemic blasts. More than 98% of acute myeloid leukaemia can be distinguished from acute lymphoblastic leukaemia by flow cytometric analysis of surface markers. Immunophenotyping is particularly important in the proper identification of minimally differentiated acute myeloid leukaemia (M0), and has enabled the identification of acute megakaryoblastic leukaemia, as well as AML co-expressing lymphoid-associated antigen.

OBJECTIVE: To evaluate the expression and patterns of commonly used immunomarkers in the diagnosis of AML by four-color multiparameter flow cytometry and to elucidate the immunophenotypic correlation with French-American-British (FAB) subtypes of AML.

METHODS: We retrospectively analyzed the immunophenotypes of 198 cases of de novo AML by using a panel of 25 monoclonal antibodies; CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD13, CD14, CD15, CD16, CD19, CD20, CD33, CD34, CD38, CD56, CD64, CD117, HLA-DR, MPO, TdT, and cCD79a. The immunophenotyping analysis of 126 peripheral blood and 72 bone marrow samples of AML cases was done using BD FACS Calibur TM and FACS canto II flow cytometers.

RESULTS: CD13, CD33 and HLA-DR were the most frequently expressed antigens; 179 (90%), 171 (86%), and 158 (80%) respectively. The panmyeloid phenotype (CD13, CD33, CD117 and MPO) were expressed in 87 patients (44%) of cases. Lymphoid-associated antigens were expressed in (36%) of cases; CD7 was the most commonly co-expressed lymphoid antigen 51(26%), followed by CD19 16(8%), and CD2 (3%). Surface and cytoplsmic CD3, CD8, CD20 and cytoplasmic CD79a were not detected in any case. CD4, CD7, CD19 and CD1a were significantly correlated with M4, M0, M2, and M5 FAB subtypes respectively.

CONCLUSION: Our data encourage the routine implementation of multiparameter flow cytometry in the diagnostic work-up of AML due to its high applicability in the detection of leukaemic cells in patients with AML. CD13 and CD33 were the most useful markers in the diagnosis of AML. CD7 and CD19 were the most commonly co-expressed T and B-associated antigens.

Key words: AML, CD13, HLA-DR, CD7, flow cytometry, FAB.

Iraqi New Medical Journal January 2018;4(1)

INTRODUCTION

Over the years, haematological flow cytometry has revealed a series of attractive features that can be recorded, as lightness, quickness, exactitude, visibility and multiplicity. Haematological flow cytometry is light because its underlying principle is solid, without any intricate conjecture, and operator involvement is trouble-free. It is quick, as first results are available within 20 minutes from the sample processing and, in an experienced laboratory, diagnosis of acute leukaemia can be provided within one hour. Flow cytometry is an exact technology,

because the analytical process is highly controlled and data are quantitatively expressed. It is visible, for the reason that great part of analysis is based upon the pattern recognition and 'eyeballing' of flow cytometry dot, density and contour plots to identify abnormalities. Finally, multiplicity is a progressively growing feature of haematocytometry, as polychromatic analysis has reached the level of six colours for routine analysis, eight for minimal residual disease studies.[1]

The characterization of acute leukaemias is based on multiparametric analysis which includes clinical features, cell morphology, genetics and immunological markers. These markers have been shown to be important for the diagnosis and prognosis of acute myeloid leukaemia (AML), particularly when the precise lineage of the blast cells cannot be defined by light microscopy morphology and cytochemistry.[2, 3, 4]

The immunophenotypic analysis of acute leukaemia by flow cytometry has become a powerful tool for proper identification of myeloid or lymphoid lineage. Although many acute leukaemias can be correctly identified morphologically with or without enzyme cytochemical analysis, immunophenotyping remains indispensable for proper identification of myeloid lineage in minimally differentiated acute myeloid leukaemia (M0),[5] and has enabled the identification of acute megakaryoblastic leukaemia,[6] as well as AML co-expressing lymphoid-associated antigen.[7] The applications of flow cytometry immunophenotyping in acute myeloid leukaemia mainly rely on the concept that even if neoplastic cells show a great similarity to normal haematopoietic precursors, they frequently display aberrant phenotypes that allow their specific identification and discrimination from normal cells, even when present at very low frequencies.[8]

In the current study we have retrospectively analyzed the immunophenotypic data from 198 de novo acute myeloid leukaemia; we attempted to 1) clarify the diagnostic value of immunophenotyping by four colour multiparameter flow cytometry in the diagnosis of AML especially (M0 subtype); 2) illustrate the immunophenotypic features of AML; 3) evaluate immunophenotyic correlation with French-American-British (FAB) subtypes.

METHODS

Setting and study design: This is a retrospective, descriptive study based on retrieving the data of 198 patients who were consecutively newly diagnosed as AML from the archives of the laboratory of flow cytometry at the bone marrow transplant Centre in Baghdad, Iraq. Those patients were diagnosed during the period from January 2014 to June 2016.

Ethical consideration: The study was done according to the standards of the Bone Marrow transplant Centre and its approval was taken.

Patients and Specimens: we used the records of the patients who were newly diagnosed as AML during the studied period. Data were retrieved with regards to some demographic factors, morphological diagnosis and assessment of their immunophenotyping patterns by four colour flow cytometry.

Morphologic Examination: All specimens were obtained and prepared for morphologic examination using standard techniques.[9] Bone marrow aspirate smears and peripheral blood specimens were air dried and stained with Leishman's stain and examined under light microscopy.

Immunophenotype Detection of Leukemia Cells: Whole blood or bone marrow aspirate collected in EDTA tube were analysed by flow cytometry using a large panel of flurochrome-labeled monoclonal antibodies. The monoclonal antibodies used in this study included fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll-protein (Per-CP), and allophycocyanin (APC) labeled CD45, CD1a, CD2, sCD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD13, CD14, CD15, CD16, CD19, CD20, CD33, CD34, CD38, CD56, CD64, CD117, HLA-DR, MPO, TdT, cCD3, cCD79a, and isotype control IgGs. CD36, CD235a and CD41a were added only when there is suspicion for the diagnosis of M6 or M7 respectively from the morphological examination. The antibody reagents and 12 mm × 75 mm Falcon capped polystyrene test tubes were provided by Becton-Dickinson Bio-science. For each tube 100µl of well-mixed, EDTA anticoagulated whole blood or bone marrow sample, and 6 µl of 4-colour direct fluorescent-labeled antibodies, which had to be fit together according to the detection protocol and the different fluo-

Table 1: Clinical and haematological characteristics of 198 patients with de novo AML at diagnosis, and partition of cases according to FAB subtypes.

General characteristics	No. (Total 198)
Median age in years (range)	38 (10 m - 85 y)
Sex (Male)	120/198
M:F ratio	1.5/1
Clinical features	
Pallor	122(62%)
Fever	99(50%)
Lymphadenopathy	34(17%)
Hepatosplenomegaly	42(21%)
Bleeding manifestation	43(22%)
Hematological features	
Median Hb level (g/dl) (range)	8 (1.3-15.6)
Median WBC count ($x10^9$/L) (range)	22 (1.6-400)
Median platelet count($x10^9$/L) (range)	42 (0-272)
Median percentage of blasts in the peripheral blood or bone marrow by flow cytometry (range)	68 (7-96)
FAB subtypes	
M0	30(15%)
M1	41(21%)
M2	9(5%)
M3	19(10%)
M4	85(43%)
M5	8(4%)
M6	5(3%)
M7	1(0.5%)

All values are numbers (%) unless otherwise indicated. WBC, white blood cells; Hb, haemoglobin; FAB, French-American-British.

rescence were added into the bottom (according to standard operating procedure of flow cytometry laboratory and the manufacturer's instruction). Samples were incubated for 15 min at room temperature in a dark place, then 2 ml of BD FACS lysing solution was added and incubated for 10 min at dark place, then the tubes were centrifuged at 1500 rpm for 5 min, supernatant aspirated, and 2 ml of BD Cell Wash solution was added to wash the cells two times. After the last wash the cell buttons in each tube were resuspended with 0.5 ml of BD CellFIX solution and subjected to data acquisition and analysis. For the detection of intracellular antigens, permeabilizing solution was added and incubated. Then MPO, cCD3, cCD79a, and nuclear TdT were added and incubated. Four-color flow cytometric analysis was performed using BD FACS Calibur TM flow cytometer (Becton-Dickinson, Bio) and FACS canto II flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA). Ten thousand events were acquired per tube to ensure the best definition of each cell population. The acquired data were analyzed using CellQuest software (Becton-Dickinson, San Jose, CA) and FacsDiva software respectively. Identification of blast cells was performed using forward scatter (FSC) versus side scatter (SSC) parameters and CD45 intensity versus SSC dot plots. An antigen was considered positively expressed when at least 20% of the gated cells expressed that antigen, however, the cutoff limit for a positive TdT, MPO, cCD3 and cCD79a expression was 10% or more.

Statistical Analysis: Analysis of data was carried out using the available statistical package of SPSS-24 (Statistical Packages for Social Sciences- version 24). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of difference of different percentages (qualitative data) was tested using Pearson Chi-square test (X^2-test) with application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05.

RESULTS

The samples consisted of 126 peripheral blood and 72 bone marrow specimens. The patients were 120 males and 78 females with a median age of 38 years, ranging from 10 months to 85 years, including 30 cases of M0, 41 cases of M1, 9 cases of M2, 19 cases of M3, 85 cases of M4, 8 cases of M5, 5 cases of M6 and one case of M7 according to FAB classification.[6] The patients' informed consents were obtained previously. Patients' characteristics at diagnosis are summarized in Table 1.

The expression of 25 antigens and the percentage of blasts positivity for each antigen of 198 de novo AML patients are presented in table 2. The percentage of positive blasts for a given positively expressed surface monoclonal antibody varied from 20% to 100% and ranged from 11% to 100% for cytoplasmic antigens (MPO and TdT). Seven markers were expressed in more than 50% of cases; namely CD13 (90%), CD33

Table 2: Pattern of phenotypic expression of 198 cases of de novo AML and the percentage of positive blasts for a given antigen.

	AML cases n=198		% of positive blasts for a given positive antigen	
Antigens	No	%	Median	Range
CD 117	143	72	63	(21-99)
CD 34	132	67	76	(21-100)
HLA-DR	158	80	83	(20-100)
CD 11b	67	34	45	(20-99)
CD 13	179	90	79	(21-99)
CD 14	34	17	47	(20-99)
CD 15	11	6	45	(26-63)
CD 16	34	17	50	(21-100)
CD 33	171	86	85	(22-100)
CD 38	152	77	61	(21-100)
CD 56	29	15	40	(23-96)
CD 64	97	49	49	(20-99)
MPO	144	73	60	(11-98)
CD 4	89	45	42	(20-99)
TdT	11	6	50	(19-100)
CD 10	4	2	40	(24-96)
CD 19	16	8	46	(21-99)
CD 1a	1	0.5	41	-
CD2	5	3	42	(21-59)
CD 5	3	1.5	44	(43-99)
CD 7	51	26	60	(23-98)

Results of immunophenotyping are given as number and percentage of positive cases, the percentage of positive blasts for certain antigen are given as a median and range.

(86%), HLA-DR (80%), CD38 (77%), MPO (73%), CD117 (72%) and CD34 (67%). CD14, CD15, CD16, CD56 and TdT were expressed in less than 20% of cases, while CD4 and CD64 were expressed in (45%) and (49%) of cases respectively (Table 2).

The expression of the four myeloid antigens (CD13, CD33, CD117 and MPO) defining the panmyeloid phenotype was analysed in the 198 patients. The leukaemic blasts of 87 patients (44%) expressed the four antigens, 159 patients (80%) were positive for two antigens (CD13 and 33). Two cases of AML (1%) were negative for all myeloid markers and they were M6 and M7 subtypes after confirmation of their diagnosis by their positivity for CD36, CD235a and CD41a respectively (Table 3).

Seventy-one (36%) of AML cases co-expressed lymphoid associated antigens; (CD1a, CD2, CD5, CD7, CD10 and CD19). T-cell and B-cell associated antigens were co-expressed in 54 patients (27%) and 17 patients (9%) respectively. Three cases (2%) have simultaneously co-expressed both T and B-cell associated markers. All AML subtypes demonstrated lymphoid associated antigens except for M7, and lymphoid antigens co-expression was most common in M2 (78% of cases) followed by M0 (67% of cases) (Table 3). CD7 was the most frequently co-expressed lymphoid marker; it was present in 49 of 198 cases (26%) (Table 2). The next most frequently co-expressed lymphoid associated marker was CD19 which presented in 16 patients (8%), followed by CD2 (3%), CD10 (2%), CD5 (1.5%) and CD1a (0.5%) (Table 2). The co-expression of surface CD3 (sCD3), cytoplasmic CD3 (cCD3), CD8, cytoplasmic CD79a (cCD79a) and CD20 were not detected in any case.

(Table 4) showed statistically significant differences in antigenic expression observed among the various FAB subtypes. There was preferential expression of CD33 among M0 (P=0.024), M1 (P=0.024), and M4 (P=0.006) (Table 4). CD117 appeared to be equally distributed among FAB subtypes with the exception of M3 (p=0.0001) and M5 (p=0.025) which showed the lowest frequencies of expression (7/19 cases and 3/ 8 cases) respectively. Stem cell antigen CD34 was strongly associated with M0 subtype with a positivity in 25 of 30 cases; (P=0.036), whereas its positivity was significantly lower in M3 (6/19); (p=0.001) (Table 3 and 4). HLA-DR was significantly associated with M4 subtype (p=0.001) and negatively associated with M3 (P=0.0001), MPO was significantly higher in M1 (39/41; p=0.0001), M3 (18/19; P=0.023) and M4 (72/85; P=0.001), while it was negatively correlated with M0 (2/30; P=0.0001), and M5 (3/8; P=0.022). TdT was significantly associated with the more immature FAB subtype M0 (P=0.0001). CD4, CD11b, CD14, and CD64 were strictly linked to M4 FAB subtype (p<0.05). CD15 presented a close association with FAB M4 and M5 subtypes (8/85; p=0.040), and (2/8; p=0.014) respectively and poorly expressed in M2 (1/9), while no expression of CD15 were detected in M0, M1, and M3. CD16 was more frequently expressed in M4 FAB subtype (23/85; p=0.001). Co-expression of CD38 showed a higher level of positivity in M4 FAB subtype (74/85; p=0.003), whereas the percentage of its positivity was significantly lower

Table 3: Immunophenotype of the 198 acute myeloid leukaemia (AML) patients according to different FAB subtypes.

Antigens	M0 n=30	M1 n=41	M2 n=9	M3 n=19	M4 n=85	M5 n=8	M6 n=5	M7 n=1
CD 117	24(80%)	34(83%)	8(89%)	7(37%)	64(75%)	3(38%)	3(60%)	0(0%)
CD 34	25(83%)	30(73%)	8(89%)	6(32%)	56(66%)	4(50%)	3(60%)	0(0%)
HLA-DR	23(77%)	34(83%)	9(100%)	5(26%)	77(91%)	8(100%)	2(40%)	0(0%)
CD 11b	5(17%)	0(0%)	1(11%)	1(5%)	55(65%)	5(63%)	0(0%)	0(0%)
CD 13	27(90%)	37(90%)	9(100%)	16(84%)	80(94%)	6(75%)	4(80%)	0(0%)
CD 14	1(3%)	0(0%)	0(0%)	1(5%)	29(34%)	3(38%)	0(0%)	0(0%)
CD 15	0(0%)	0(0%)	1(11%)	0(0%)	8(9%)	2(25%)	0(0%)	0(0%)
CD 16	4(13%)	0(0%)	3(33%)	2(11%)	23(27%)	2(25%)	0(0%)	0(0%)
CD 33	22(73%)	31(76%)	9(100%)	18(95%)	80(94%)	7(88%)	4(80%)	0(0%)
CD 38	26(87%)	29(71%)	7(78%)	6(32%)	74(87%)	8(100%)	2(40%)	0(0%)
CD 56	3(10%)	5(12%)	3(33%)	1(5%)	14(16%)	3(38%)	0(0%)	0(0%)
CD 64	1(3%)	1(2%)	0(0%)	12(63%)	76(89%)	6(75%)	1(20%)	0(0%)
MPO	2(7%)	39(95%)	9(100%)	18(95%)	72(85%)	3(38%)	1(20%)	0(0%)
CD 4*	10(33%)	6(15%)	2(22%)	4(21%)	62(73%)	4(50%)	1(20%)	0(0%)
TdT	7(23%)	3(7%)	0(0%)	0(0%)	1(1%)	0(0%)	0(0%)	0(0%)
CD 10	1(3%)	0(0%)	0(0%)	0(0%)	3(4%)	0(0%)	0(0%)	0(0%)
CD 19	2(7%)	5(12%)	3(33%)	1(5%)	5(6%)	0(0%)	0(0%)	0(0%)
CD 1a	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(13%)	0(0%)	0(0%)
CD 2	2(7%)	0(0%)	0(0%)	1(5%)	2(2%)	0(0%)	0(0%)	0(0%)
CD 5	2(7%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(20%)	0(0%)
CD 7	19(63%)	10(24%)	4(44%)	0(0%)	16(19%)	2(25%)	0(0%)	0(0%)
CD 41a	NT	NT	NT	NT	NT	NT	NT	1(100%)
CD 36	NT	NT	NT	NT	NT	NT	2(40%)	NT
CD 235a	NT	NT	NT	NT	NT	NT	4(80%)	NT

Results of immunophenotyping are given as number and percentage of positive cases for each FAB subtype. CD, cluster of differentiation, NT; not tested.
*CD4 was studied but was not considered as a lymphoid specific antigen, as it has frequently reported in normal and leukaemic myeloid cells.[10]

in M3 FAB subtype (6/19; p=0.0001). CD56 was co-expressed in FAB subtypes M0, M1, M2, M3, M4, and M5, but it did not correlate with any of the FAB subtypes at a significant level (p value > 0.05) (Table 3 and 4).

Co-expression of CD19 was found in the subtypes M0, M1, M2, M3, and M4 with preferential expression in M2 (3/9 of cases; p=0.004). CD7 co-expression observed in FAB subtypes M0, M1, M2, M4, and M5 and occurred more frequently in M0 (19/30 of cases; p=0.0001), and negatively associated with M3 (0/19; P=0.007). CD1a was exclusively co-expressed in FAB subtype M5 (1/8 of cases; p=0.0001), CD5 showed a statistically significant association with FAB subtypes M0 and M6 (2/30 of cases; p=0.012) and (1/5; p=0.001) respectively (Table 3 and 4). No statistically significant correlations were observed between FAB subtypes and CD2 and CD10 co-expression, (p value > 0.05).

DISCUSSION

Immunophenotyping by flow cytometry (FCM) provides a rapid and early recognition of AML cases that overcomes uncertainty of the morphologic diagnosis or confusion resulting from an initial negative cytogenetic result during the diagnostic process. Thus implementation of immunophenotyping by flow cytometry as a part of the diagnostic work-up of AML offers an effective means of providing the accurate diagnosis.

In this study, we retrospectively analysed the immunophenotype of 198 cases of de novo AML. CD13 followed by CD33 were the most frequently expressed myeloid-associated antigens in agreement with Legrand et al,[11] Baer et al,[12] and Gujral et al;[13] however, khalidi et al,[14] Zheng et al,[15] and kaleem et al[16] have reported that

Table 4: Statistically significant FAB subtype immunophenotypic correlation.

CD marker	FAB subtype	No. of positive cases		Type of AML compared	No. of positive cases	P value
CD117	M3	7/19	VS	All other AMLs	136/179	0.0001
	M5	3/8	VS	All other AMLs	140/190	0.025
CD 34	M0	25/30	VS	All other AMLs	107/168	0.036
	M3	6/19	VS	All other AMLs	126/179	0.001
HLA-DR	M3	5/19	VS	All other AMLs	153/179	0.0001
	M4	77/85	VS	All other AMLs	81/113	0.001
CD11b	M4	55/85	VS	All other AMLs	12/113	0.0001
CD14	M4	29/85	VS	All other AMLs	5/113	0.0001
CD15	M4	8/85	VS	All other AMLs	3/113	0.040
	M5	2/8	VS	All other AMLs	9/190	0.014
CD16	M4	23/85	VS	All other AMLs	11/113	0.001
CD 33	M0	22/30	VS	All other AMLs	149/168	0.024
	M1	31/41	VS	All other AMLs	140/157	0.024
	M4	80/85	VS	All other AMLs	91/113	0.006
CD 38	M3	6/19	VS	All other AMLs	146/179	0.0001
	M4	74/85	VS	All other AMLs	78/113	0.003
CD64	M4	76/85	VS	All other AMLs	21/113	0.0001
MPO	M0	2/30	VS	All other AMLs	142/168	0.0001
	M1	39/41	VS	All other AMLs	105/157	0.0001
	M3	18/19	VS	All other AMLs	126/179	0.023
	M4	72/85	VS	All other AMLs	72/113	0.001
	M5	3/8	VS	All other AMLs	141/190	0.022
CD4	M4	62/85	VS	All other AMLs	27/113	0.0001
TdT	M0	7/30	VS	All other AMLs	4/168	0.0001
CD 19	M2	3/9	VS	All other AMLs	13/189	0.004
CD 1a	M5	1/8	VS	All other AMLs	0/190	0.0001
CD 5	M0	2/30	VS	All other AMLs	1/168	0.012
	M6	1/5	VS	All other AMLs	2/193	0.001
CD 7	M0	19/30	VS	All other AMLs	32/168	0.0001
	M3	0/19	VS	All other AMLs	51/179	0.007

CD33 followed by CD13 were the most commonly expressed myeloid-associated antigens. In this study, the diagnostic value of panmyeloid reagents CD13 and CD33 for the recognition of AML cases was high (97%) which is slightly higher than the level of positivity reported (94%) by Creutzig et al.[17] From our initial results, we recognized that the use of antibodies against these two antigens is probably sufficient for an initial screening panel of acute leukaemia. The addition of CD11b and CD16 may help to confirm the myeloid lineage in cases where CD13 or CD33 were negative. The next most commonly expressed myeloid markers were MPO and CD117 in line with Zheng et al[15] and Legrand et al.[11] On the other hand in their report, Thalhammer-Scherrer et al[18] found that CD33, MPO, and CD13 were the most frequently expressed myeloid markers.

CD13 was expressed in 90% of cases of AML as reported by Reading et al[19] and Venditti et al,[20] in contrast, Casanovas et al[21] have reported a lower frequency (72%). The expression of CD13 was almost universal in AML M0 to M6, and it's in agreement with Kaleem et al,[16] while Stasi et al[22] reported a universal expression of CD13 in AML; M1 to M5 subtype. CD33 expression in the current study was 86%, in concordance with Neame et al[3] who observed a positivity for CD33 in 87% of AML cases. However, literature has

shown a result as high as 96%[15] and as low as 79%.[23] CD33 was more frequently expressed in M0 (P=0.024). On the contrary to our findings, Creutzig et al[17] found a negative correlation between CD33 expression and M0 FAB subtype.

Detection of MPO is usually made by cytochemistry[13,14,24] and less frequently by indirect immunofluorescent assay.[25] use of flow cytometry in detection of MPO is reported scarcely in literature.

MPO was positive in 73% of AML cases and was exactly as reported by Legrand et al.[11] In contrast, Zheng et al[15] observed a higher percentage of positivity for this marker,81%. The expression of MPO was significantly low in M0 (P=0.0001), and M5 (P=0.022), in contrast to Venditti et al,[20] and Stasi et al[25] who reported a positive MPO in all their AML cases. We observed that CD13 and CD33 are more reliable reagents than MPO in distinguishing AML-M0 from other FAB subtypes, this remark is reinforced by the evidence that 93% of AML-M0 cases were MPO negative, whereas only one case of M0 was negative for both CD13 and CD33.

We found that 72 % of de novo AML expressed CD117. Other investigators have reported that CD117 expression is varied from 21% to 82% of AML cases.[11,13-16 18, 20, 23, 26-27] in many of these studies the expression of CD117 is between 73 -75 % [11, 13, 16, 27] which is consistent with our finding. CD117 appeared to be statistically low in FAB subtypes M3 (p=0.0001) and M5 (p=0.025), which is inconsistent with khalidi et al[14] who reported a statistically significant low expression of this marker in M3 FAB subtype and in concordance with Sperling et al[28] who found a very low percentage of positivity for CD117 in M5 subtype; 12% of cases. On the other hand, Dong et al[29] have demonstrated that 95 % of M3 has a positive CD117.

HLA-DR was positive in 80% of our AML cases; it was at the upper end of the reported range, 70%-89%.[11,12,14,15,17,18,20,23,28,30] Creutzig et al[17] found a similar percentage. HLA-DR expression was significantly high in M4 FAB subtype (p=0.001), in concordance with Thalhammer-Scherrer et al[18] who found a positive correlation between HLA-DR expression and AML subtypes with monocytic differentiation. HLA-DR was negatively associated with M3 (p=0.0001). Many studies [13, 14, 16-18, 29] have reached to a similar result in the relation of the expression of HLA-DR and M3 FAB subtype.

Expression of CD34 was found in 67 % of our cases of AML; in the similar range shown by others.[11, 12, 22] However, the literature has reported a variable percentages from 42-68%. [11, 12, 14-23, 28] CD34 showed a preferential expression in M0 FAB subtype (P=0.036), and it was significantly low in M3 FAB subtype (p=0.001), this is consistent with the findings of Kaleem et al[16] and Venditti et al.[20]

CD11b was positive in 34% of AML cases; this frequency was very close to that reported by Zheng et al[15] which was 33%. On the contrary, Bradstock et al[23] observed a relatively higher percentage (41%). Venditti et al[20] has found that CD11b was strictly linked to M4 AML (p=0.0001).

In our study, CD14 and CD15 were expressed in 17%, and 6% of AML cases respectively which is in the lower end of the reported range (11%-82%).[12,14-16, 18] This relatively low level of CD15 in particular is of uncertain cause, but may be related to the known epitopic heterogeneity of this antigen.[23]

We found that CD14 and CD15 have a close associated with M4 AML (p value of 0.0001 and 0.040 respectively). Neame et al[3] and Kaplan et al[24] have shown similar results. We have also found that CD15 has a statistically significant association with FAB subtypes M5 (p=0.014) a similar finding to that of Thalhammer-Scherrer et al[18] and Stasi et al.[22]

CD16 was seen in 17 % of our cases of AML. It is relatively similar to the 20 % reported by Krasinskas et al[31,] but higher compared to the 2 % reported by Baer et al.[12] CD16 was more frequently expressed in M4 FAB subtype (p=0.001) as reported by Krasinskas et al. [31]

CD4 was expressed in 45% of AML cases. A wide range of CD4 positivity (14%-72%) was reported in literature.[12, 14-17, 19, 20] Our finding was relatively similar to that of khalidi et al.[14] CD4 was found predominantly in M4 FAB subtype, (P=0.0001). In accordance with Venditti et al.[2] CD64 was detected in 49% of AML cases, which was very close to the figure demonstrated by Baer et al,[12] Our study has shown a statistically significant correlation between CD64 and M4

AML (P=0.0001) in agreement with Krasinskas et al.[31] This is explainable as CD64 reacts usually with cells of monocytic origin.[33]

In our series, CD56 was expressed in only 15% of AML cases and it did not show any statistically significance correlation with any FAB subtypes (p>0.05), in concordance with khalidi et al.[14] However, Raspadori et al.[34] found that it was more frequently associated with M5 FAB subtype.

TdT marker was expressed in 6 % of our cases of AML. Literature has reported a wide range of expression between 7-52 %, [7,15-18,21,22,35] so our result is similar to that reported by Zheng et al,[15] but far lower than the 52 % reported by Cuneo et al.[7] TdT expression was tightly linked to M0 FAB subtype (p=0.0001). This finding is in line with previous reports documenting a higher incidence of TdT in poorly differentiated AML.[15-17, 20, 22] Co-expression of CD38 was detected in 77% of AML cases which is relatively similar to the figure found by Marinov et al,[36] 75%. On the contrary, Baer et al[12] reported a higher frequency, 96%.

Our study confirmed the phenomenon of lymphoid antigen co-expression in AML; 36% of AML cases have co-expressed lymphoid markers (CD1a, CD2, CD5, CD7, CD10, and CD19), which is within the range of 20-54 % that was noted by other investigators.[14, 18, 19, 35, 37-39] Our findings of lymphoid antigens co-expression is lower than that of khalidi et al [14] and Reading et al[19] who have reported 48% and 54% respectively; whereas , Jha et al [39] have reported a close figure to ours.

CD7 was the most frequently co-expressed lymphoid marker in this series in agreement with many studies.[11, 12, 15, 17-19, 27, 38-40] CD7 was co-expressed in 26% of cases of AML. A percentage that was identical to what was reported by Reading et al[19] and Bahia et al,[38] but higher than the 8.8% reported by Thalhammer-Scherrer et al[18] and lower compared to the percentage (37%) reported by Legrand et al.[11] CD7 was significantly correlated with FAB subtype M0 (P = 0.0001) in agreement with Venditti et al[35] whereas khalidi et al[14] did not find such an association. Absence of CD7 co-expression in M3 FAB subtype in this report was in accordance with the findings of Bahia et al.[38]

CD19 was the next most frequently co-expressed lymphoid-associated antigen, CD19 was co-expressed in 8% of AML cases which was close to the figure seen by Bahia et al,[38] in contrast to the higher results,16%, reported by Legrand et al[11] and the lower finding (4%) showed by Al-Mawali et al. [40] A preferential co-expression of CD19 in M2 FAB subtype at a significant level (p=0.004) was observed, which was similarly reported by Kita et al[41] On the other hand, Creutzig et al[17] did not find such correlation.

CD5 and CD10 were detected in less than 5% of AML cases in accordance with the results of Zheng et al,[15] while Thalhammer-Scherrer[18] did not show any positivity for CD10 in their AML cases. Reading et al[19] demonstrated a higher percentage of positivity for CD5 (10%) in their series.

The current study showed that CD2 co-expressed in 3% of AML cases which was identical to the figure found by Thalhammer-Scherrr et al,[18] while Legrand et al[11] found a higher incidence, 18%.

The co-expression of surface and cytoplasmic CD3, CD8, cytoplasmic CD79a and CD20 was not detected in any AML case, this is in accordance with Legrand et al[11] who found no co-expression for cCD3, CD8, and CD20 in all AML cases tested for those markers in their series. Thalhammer-Scherrr et al[18] demonstrated a low percentage of positivity for (cCD79a) in AML in their studies (2%). Some differences in the percentage of positivity occasionally encountered in this series compared to previously mentioned publication may be related to technical issues or could be due to differences in individual laboratory protocols or due to ethnical variation.

CONCLUSIONS

Flow cytometry is a very useful tool for the diagnosis and subclassification of AML, due to its high applicability in the detection of leukaemic cells in patients with AML and its capability to discriminate various FAB subtypes from each other in the vast majority of cases; therefore, a swift

flow cytometric analysis should be considered as an independent component of AML work up complementary to cytogenetic testing and cytochemistry. CD13 and CD33 were the most frequently expressed myeloid antigens. Thirty-six percent of AML cases co-expressed lymphoid–associated antigens. CD7 and CD19 were the most commonly co-expressed T and B-associated antigens. CD4, CD7, CD19 and CD1a were significantly correlated with M4, M0, M2, and M5 FAB subtypes respectively. There was an absence of CD7 expression in all cases of M3 FAB subtypes. The co-expression of surface and cytoplasmic CD3, CD8, cytoplasmic CD79a and CD20 was not detected in any of the 198 AML cases.

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Abbreviation list: Acute myeloid leukaemia (**AML**), Allophycocyanin (**APC**), Ethylenediaminetetraacetic acid (**EDTA**), Flow cytometry (**FCM**), Forward scatter (**FSC**), Fluorescein isothiocyanate (**FITC**), French-American-British (**FAB**), Haemoglobin (**Hb**), Microliter (**µl**), Millilitre (**ml**), Minute (**min**), Phycoerythrin (**PE**), Peridinin chlorophyll-protein (**Per-CP**), Round per minute (**rpm**), Statistical Packages for Social Sciences- version 24 (**SPSS-24**), Versus side scatter (**SSC**), White blood cells (**WBC**)

Conflict of interest: Author has nothing to declare.

Funding: Authors received no funds to complete this report a part from self funding.