RESEARCH ARTICLE

Clinical Significance of Co-expression of Aberrant Antigens in Acute Leukemia: A Retrospective Cohort Study in Makah Al Mukaramah, Saudi Arabia

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Abstract

Background: Aberrant phenotypes in acute leukemia have variable frequency and their prognostic and predictive relevance is controversial, despite several reports of clinical significance. Aims: To determine the prevalence of aberrant antigen expression in acute leukemia, assess clinical relevance and demonstrate immunophenotype-karyotype correlations. Materials and Methods: A total of 73 (40 AML and 33 ALL) newly diagnosed acute leukemia cases presenting to KAMC, Kingdom of Saudi Arabia, were included. Diagnosis was based on WHO criteria and FAB classification. Immunophenotyping by flow cytometry, conventional karyotyping and fluorescence *in situ* hybridization for gene rearrangements were performed. Results: Aberrant antigens were detected in 27/40 (67.5%) of AML and in 14/33 (42.4%) in ALL cases. There were statistically significant higher TLC in Ly+ AML than in Ly-AML (p=0.05) and significant higher blast count in ALL with aberrant antigens at presentation and day 14 (p=0.005, 0.046). There was no significant relation to clinical response, relapse free survival (RFS) or overall survival (p>0.05), but AML cases expressing ≥2 Ly antigens showed a lower median RFS than those expressing a single Ly antigen. In AML, CD 56 was expressed in 11/40. CD7 was expressed in 7/40, having a significant relation with an unfavorable cytogenetic pattern (p=0.046). CD4 was expressed in 5/40. CD19 was detected in 4/40 AML associated with M2 and t (8; 21). In ALL cases, CD33 was expressed in 7/33 and CD13 in 5/33. Regarding T Ag in B-ALL CD2 was expressed in 2 cases and CD56 in 3 cases. Conclusions: Aberrant antigen expression may be associated with adverse clinical data at presentation. AML cases expressing ≥2 Ly antigens may have shorter median RFS. No specific cytogenetic pattern is associated with aberrant antigen expression but individual antigens may be related to particular cytogenetic patterns. Immunophenotype-karyotype correlations need larger studies for confirmation.

Keywords: Aberrant antigen - flow cytometry - AML - ALL - prognosis - cytogenetics

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Introduction

The latest WHO 2008 classification of acute leukemia uses morphology, immunophenotype, genetics and clinical features to define clinically significant disease entities (Vardiman et al., 2009). Distinction between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) is extremely important and flowcytometry (FCM) is very instrumental in this. Malignant blasts often have an abnormal phenotype that allows distinction from normal immature cells (Al-Mawali et al., 2008). One of these abnormalities is the occurrence of aberrant phenotypes (or anomalous expression) which is defined by the co-expression of markers usually not present on cells of that particular lineage (Ossenkoppele et al., 2011) e.g. lymphoid antigen positive AML (Ly+AML) and myeloid antigen positive ALL (My+ALL) not classified as mixed phenotype leukemia (MPL). Aberrant antigen expression is reported to have variable frequency (Voskova et al., 2003; Vitale et al 2007; Zhang et al., 2012; Novoa et al., 2013) most commonly CD7 (Chang et al., 2007), CD9, CD19 and CD56 in AML cases (El-Sissy et al., 2006) as well as CD13 and CD33 in ALL (Liu et al., 2007; Suggs et al., 2007) and their prognostic and predictive relevance is controversial (Bhushan et al., 2010). However, several reports discussed their clinical significance, but most of them were not comprehensive (Putti et al., 1998; Venditti et al., 1998; Ogata et al., 2001).

In AML, characteristic antigens have been related to particular morphological FAB subtypes and associated with the presence of recurrent genetic abnormalities (Bagg, 2007), such as AML-M2 with t(8;21) that shows

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aberrant expression of lymphoid markers include CD19 and CD56 (Khoury et al., 2003), another one is co-expression of CD2 in M4E with inv(16) or t(16; 16), although not specific for this type of AML (Dunphy, 1999; Medeiros et al., 2010) and M5 with t(9;11) is reported to have high expression CD56 (Graf et al., 2005; Wang et al., 2005). Regarding ALL, t (9; 22) was reported to be more frequently observed in My+B ALL than in My- B ALL (Wu et al., 2007).

One of the newly emerging importance of immunophenotypical aberrancies using FCM is the detection and quantification of minimal residual disease (MRD) for providing prognostic information, and make use of such aberrancies in routine management of patients to guide therapy (Kern et al., 2008; Buccisano et al., 2009). There is much to be discovered about the clinical characteristics and significance of co-expression of two or more aberrant lineage leukemia markers (Bhushan et al., 2010).

Materials and Methods

To determine the prevalence of aberrant antigens in acute leukemia, demonstrate immunophenotype-karyotype correlations, detect any unique clinical features at presentation related to aberrant antigen expression in our center; King Abdulla Medical City (KAMC), Makkah, Saudi Arabia (KSA) and correlate their expression with treatment out come.

Study design

Patients and clinical samples: A total of 79 untreated newly diagnosed acute leukemia patients presenting to KAMC, Makkah, KSA between October 2010 to Feb 2013. Six cases were diagnosed as mixed phenotype leukemia (MPL); 4cases M/B and 2 cases M/T were excluded and 40 AML and 33 ALL cases were included in this study. Diagnosis was based on WHO criteria in addition to FAB classification. The study abides by the rules of institutional review board- approval protocol. All cases had representative bone marrow aspiration together with trephine core biopsy specimens for evaluation, EDTA peripheral blood or bone marrow aspirate specimens for flowcytometry analysis of surface and cytoplasmic markers, and heparinised sample for cytogenetic study.

Immunophenotyping: it was performed using BD-FACS-Canto II System (BD- Bio Science) and reagent system (BD- FACS Setup) as previously described (Ludwig et al., 1998) using a panel of monoclonal antibodies that included; B cell lineage markers (CD10, CD19, CD20, CD22, cyt CD79a, Kappa and Lambda light chains, surface and cyt IgM); T cell lineage markers (CD2, surface and cytoplasmic CD3, CD5, CD7, CD4, CD8, CD1 a), myeloid markers (CD13, CD14, CD15, CD33, CD64, CD117, CD11c, cyto MPO) and miscellaneous markers (CD34, CD45, CD56, CD38, HLA DR, TdT).Cell populations were designated as positive for a particular surface antigen if expressed in ≥ 20% of blasts events (stained beyond an appropriate isotypic cutoff) and for intracellular antigen 10%.

Cytogentic analysis: Conventional karyotypic analysis was performed on metaphase cells using standard culturing and banding techniques, results were reported in accordance to the International System for Human Cytogenetics Nomenclature (ISCN, 1985). Fluorescence in situ hybridization for gene rearrangements was performed on interphase nuclei.

Statistical methods

Data was analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation for variables with normal distribution or median and range as for variables deviating from normal distribution appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Survival analysis was done using Kaplan-Meier method and comparison between survival curves was done using log-rank test. A p-value<0.05 was considered significant.

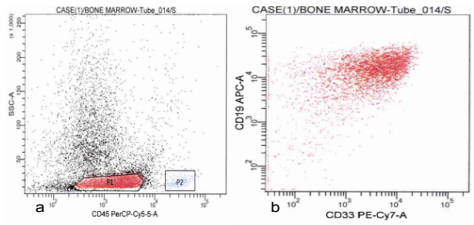

Figure 1. FCM Dot Blots of a Representative B-ALL Sample. Gating using the CD45 protocol, P1 represents the dim CD45 cells; blasts and P2 represents the bright CD45 cells; residual normal lymphocytes (a). Dot blot represents coexpression of CD33 and CD19 on the gated cells (b)

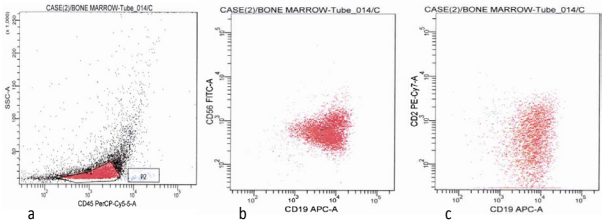

Figure 2. FCM Dot Blots of a Representative AML Sample. Gating by CD45 protocol, P1 represents the CD45 cells; blasts and P2 represents the bright CD45 cells; residual normal lymphocytes (a). Dot blot represents coexpression of CD56 and CD19 on the gated cells (b). Dot blot represents coexpression of CD2 and CD19 on the gated cells (c)

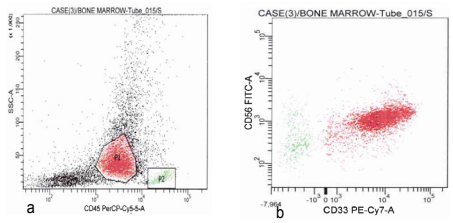

Figure 3. FCM Dot Plot of Arepresentitive AML Case. Gating using the CD45 protocol, P1 represents the dim CD45 cells; myeloblasts and P2represents the bright CD45 cells; residual normal lymphocytes (a). dot blot represent coexpression of CD33 and CD56 on the gated cells (b)

Results

This study included 40 AML and 33 ALL (26 B and 7 T-ALL) newly diagnosed cases; their clinical data at presentation are shown in Table1.

AML results

The AML cases were classified according to FAB classification into: M1(10 cases), M2(9 cases), M3(3 cases), M4(12 cases), M5(4 cases), M6(1 case) and M7(1 case). Aberrant lymphoid antigens (Ly+) were detected in 27/40 (67.5%). Among the Ly+AML, 9/27 cases (33.3%) expressed ≥2 Ly antigens; 2 cases expressed 3 Ly antigens; one was M1 expressing CD2, CD7 & CD9 and the other was M2 expressing CD56, 19&9 and 7 cases expressed 2 Ly antigens. 5/9 (55.5%) expressing ≥2Ly antigens cases died due to disease complication, 2/9 (22.2%) relapsed and 1/9 (11.1%) did not achieve complete remission (CR). The remaining cases (18) expressed single Ly antigen, 7/18(38.8%) died and 2(11.1%) relapsed but alive with disease. There was no statistical significant difference between Ly+and Ly- AML regarding FAB subtypes, cytogenetic analysis, clinical, or hematological findings at presentation except for significantly higher TLC, median (range) 25.3 (0.9-397) in the Ly+AML than the Ly-AML 6.0(0.7-105.8) (p=0.05). There was no statistical significant difference between Ly-AML and Ly+AML cases regarding overall survival (OS) or relapse free survival (RFS) (p=0.75 and 0.21 respectively), the median RFS±SE in the cases that expressed ≥2 Ly antigens was 35.9±14.4; lower than cases that expressed one lymphoid antigen 92.9±0.2.while the median OS±SE were comparable 69.3±49.8 for one lymphoid antigen expression versus 58.1±35.6 for cases that expressed ≥2 Ly antigens.

CD56 was expressed in 11/40 (27.5%) cases, and its distribution within FAB subtypes and relation with cytogenetics are shown in Table 2. Out of these 11 patients 3 (27.2%) cases could not achieve complete remission from the 1st induction, another one relapsed and 4 (36.4%) cases died before the end of this study, but this finding did not reach statistical significance difference between CD56+and CD56-AML cases regarding OS (p=0.726).

CD7 was expressed in 7/40 cases (17.5%), 6/7 belongs to M1 subtype representing the most frequent Ly antigen in M1 and were also coexpressing CD34 the remaining case was M2. Their cytogenetic pattern showed MK with other cytogenetic abnormalities [46XY, t(4;19), 7q deletion], complex karyotype [47XX, t(10;11) (p12;q13),+4, add(8) (p23),+7(by FISH)] and [47XX,+11(trisomy MLL by FISH)], the remaining cases did not show any recurrent translocations by FISH, this cytogenetic pattern represents a statistical significant relation between CD7expression in AML and unfavorable cytogenetic pattern (p=0.046). There was no statistical significant difference between CD7+AML and CD7- AML regarding CR or OS (p=0.68, 0.07 respectively).

CD4 is a differentiation marker in monocytic AML (Krasinskas et al., 1998) so it was considered as aberrant antigen only when expressed in cases other than M4 or M5, considering this fact, CD4 was expressed as aberrant antigen in 5/40(12.5%), their cytogenetics pattern and FAB subtypes are shown in Table 3. CD5 was expressed in one M2 case with dysplasia [46XY, 20q-]. CD2 was expressed in 2/40 (5%) cases; M1 [47XX,+11] and M3 [46XY, t (15; 17)].

Regarding the expression of B cell antigens in AML cases, CD19 was expressed in 4/40 cases (10%), 3 cases were M2 and their cytogenetic pattern was: [47XY, t (8; 21)+8], [46XX t(8;21)] and [46XY t(8;20;21) & inv 16] and it is worthy to notice that they were co expressing CD56 and the 4th case was M5 with normal karyotype[46 XY]. CD9 was done in only 8 cases within this cohort-as

Table 1. Clinical Criteria of the Studied Group

Characteristics		AML cases (40)		ALL cases (33)	
Median age at diagnosis (range), years					
		47	(14-70)	18	(14-51)
Sex	Male	26		19	
	Female	14		14	
Initial clinical features					
	Lymphadenopathy	8	(20%)	10	(30.3%)
	Hepatosplenomegaly	9	(22.5%)	8	(24.2%)
	Weight loss and fatigue	27	(67.5%)	15	(45.4%)
	Fever	19	(47%)	13	(39.4%)
	Bone pain	3	(7.5%)	6	(18.2%)
Hematological findings median (range)					
	TLC ($\times 10^9$/L)	15.6	(0.7-397)	14.9	(1.9-397)
	Hemoglobin (g/L)	8	(3-14)	8.6	(2.7-13.5)
	Platelets ($\times 10^9$/L)	38	(9-223)	65	(12-449)
	BM blasts	76	(20-97)	80	(25-99)
	LDH (U/L)	396	(102-4953)	365	(89-10544)
Cytogenetics*					
Favorable: 11 [t(8;21):4,t(15;17):3 & inv 16:4]		**Favorable: 4** [Hyperdiploidy >50, 1 and low hyperdiploidy:4]			
Unfavorable: 10 [monosomy:2, MK with other cytogenetic abnormalities:6, 11q23:2]		**Unfavorable:9** [t(9;22):3,t(1;19): 2, 11q23:2, cymc:1& 9p del:1]			
Intermediate: 9 [normal karyotype :7, trisomy 8: 2]		**undetermined:14** [normal:9,complex:3,t(10;14):1 &t(6;21):1]			

*The number of cases mentioned according to available data; **MK monosomy karyotype

Table 2. CD56 Expression in AML Group: Relation with Cytogenetics and FAB Subtypes

FAB subtype	Other aberrant antigen	Cytogenetics*	Fate
M1 (3/10)	CD7	**Failed	relapsed
	CD7	**Failed	alive
	CD4	46 XY,+21&5q minus	died
M2 (4/9)	CD19	47 XY+8, t(8;21)	alive
	CD9,CD19	46 XY, t(8;20;21)&inv 16	died
	CD9	46 XX, t(8;21)	died
	none	49XX,5q-,-7(by FISH)],+8,+19,+22	died
M4 (2/12)	None	**Failed	died
	CD4	46XY	alive
M5 (1/4)	none	46, XY	died
M6 (1/1)	none	**Failed	alive

*cytogenetics results are obtained from both conventional karyotype and FISH, **Failed Karyotype due to failed metaphase and no recurrent translocations detected by FISH

Table 3. Aberrant CD4 Expression in AML Group: Relation with Cytogenetics and FAB Subtypes

FAB subtype	CD4 (5/40)	Cytogenetics	Fate
M1	1	46XY, +21 & 5q-	died
M2	3	46XY	Relapsed & died
		47XY, +8, -7 &5q-	No CR & died
		46 XY, t(8;21)	alive
M7	1	44XY,-5,-12,7q-,12q-,t(7;12)	No CR & died

Table 4. Aberrant Antigen Expression in ALL Group: Relation with IPT, Cytogenetics and Patient Fate

IPT no (%) 14/33(42.4%)	Aberrant antigen	*Cytogenetics	Fate
Pro-B	CD2,56 & 13	46XY	alive
2/3(66.7%)	CD2	46XY	alive
Pre-B	CD15	46XY, t(1;19) / (PBX1 / TCF3)	relapsed
2/6 (33.3%)	CD14,33	**Failed	alive
Common-B	CD56	46XY, t(9;22) /	alive
5/16 (31.25%)		PCR/ABL fusion protein(210)	
	CD15,33,64	48X,-X, del(12),+21,12p del,	relapsed
	CD13	66XX[near triploidy] /FISH	missed
	CD13,33, 56	47,XX,t(1:6) ,+8, +9, t(9:13), i(9),del(12)	relapsed
	CD13	**Failed	relapsed
	CD33	47 XY,+ 8	relapsed
Precursor T	CD33	46XX, t(6;21) / ETV6/RUNX	alive
5/6(83.3%)	CD33	45 XX, 9p del	alive
	CD33	46,XX,16(p21) del., 12 del., monosomy7,+17	alive
	CD13	48XY, +4 & +21	alive

*Cytogenetics results are obtained from both conventional karyotype and FISH, **Failed Karyotype due to failed metaphase and no recurrent translocations detected by FISH

this marker was not introduced early in our panel- 6/8 (75%) were expressing CD9 that was distributed between different FAB subtypes and their karyotype was; M1 case had+11, M2 case had t(8;20;21), 2 M3 cases had t(15;17) and 2 M4 cases one had inversion 16 and+22 and the other one failed karyotype. CD22 was expressed in 3cases distributed in M1, M2 and M4 subtypes, recurrent translocations was not detected by FISH in any of them.

ALL results

Aberrant antigens was expressed in 14/33(42.4%) cases, 12/33(36.4%) expressed myeloid antigens (CD13,33,14,15&64), 4/33 (12.1%) expressed T- antigens (CD2&56) and both T&My antigens were expressed in combination in 2 B-ALL cases, the relation with cytogenetics and IPT are shown in Table 4. Among the ALL cases with aberrant antigens, 5/33 (15.1%) expressed≥2 antigens; 3 cases expressed 3 aberrant antigens, one case expressed 2 My antigens and the remaining cases expresses single aberrant antigen either My or T in B-ALL .

On comparing the ALL group with aberrant antigen expression with the group without, there was a statistically significant higher blast count at presentation, median (range) 90 (60-99) versus 65(25-95), p=0.005 and higher blast % at day 14 after induction chemotherapy (IC) 2 (0-25) versus 1(1-4), p=0.046. However there was no difference in achieving CR at day 28, OS or RFS (p>0.05).

The most frequent My antigen expressed was CD33 in 7/33 (21.2%) followed by CD13 in 5/33(15.1%), then CD15 expressed in 2 cases (6%) and both CD14 and CD64 expressed once (Table4). Aberrant antigens were detected in the less mature progenitor in T ALL, they were expressed in 5/6 (83.3%) of the precursor T-ALL supported by a statistically significant higher frequency of CD7 expression (p 0.025) together with failure of CD1. Otherwise no statistical significance detected with other CD antigens including the early antigens CD117, CD34 or CD58 in B ALL. With regards to T antigen expression in B-ALL, CD2 was expressed in 2 pro B and CD56 was expressed in 3 cases; one pro, and 2 common ALL cases.

Within the group that expressed aberrant antigen, 4 cases had hyperdiploidy one case was near triploidy (66-79 chromosomes (CS)), and it express CD13 the other 3 cases were low hyperdiploidy (47-49 CS) (Anjali et al., 2011), 2 of them (66.7%) express CD13 and lost CD45; all the hyperdiploidy cases expressed CD34. Cytogenetigc abnormality involving AML gene, either t(12;21) or trisomy or tetrosomy of AML gene was detected in 5 cases out of the whole studied group, 3 of them expressed CD10. The case that had t (12; 21) (AML/TEL) expressed CD15, 33, 64 and CD34. None of the 3 cases that had t (9; 22) (BCR/ABL) expressed My antigen, only one of them expressed CD56. However, three cases had monosomy, trisomy and tetrasomy ABL express CD13 in 2cases and CD33 in the third one. t (1; 19) was detected in 3 cases two of them (66.6%) lost CD34.

Discussion

Progress in the management and understanding of acute leukemia can only be obtained if these diseases are thoroughly investigated, both clinically and with a series of biological tools. This will make possible the identification of prognostic factors and of useful markers for the follow-up of patients in remission (Hur et al., 2001).

The frequency of aberrant expression of one or more lymphoid lineage markers was 67.5% in AML cases which was higher than that reported by earlier studies 23.9% (Xu et al., 2003), 34.3% (Bahia et al., 2001), even it is higher than what previously reported by El-Sisy et al.(2006) in a study done in King Fahd Medical City, Riyadh, KSA, where they detect aberrant lymphoid antigens in 47%, but more close to Jiang et al. (2010) who indicated that 56.5% had cross-expressing lymphoid and myeloid antigens. This higher frequency may be related to environmental changes and accumulation of biological defects. This higher frequency was of a great value in our lab to assess minimal residual disease and follow up of our patients. However, the implication of minimal residual disease assessment in AML is still under investigations.

In the current study, we could not find a solid relation between aberrant antigen expression and OS or RFS, in agreement with others who stated that RFS of AML

patients with cross-expressing antigens had no significant difference when compared with Ly(-) AML patients (Jiang et al.,2010) .The median RFS in the cases that expressed ≥2 Ly antigens was lower than that expressed one lymphoid antigen and they had many events; 5 deaths, 2 relapses and one did not achieve CR this may suggests abnormal hematopoietic differentiation with more aberrant expression of antigens of different lineages on leukemic cells that may be related to bad prognosis.

We found a significantly higher TLC in the Ly+AML than the Ly-AML (p 0.05), up to our knowledge no such data was reported before which could be considered as a bad prognostic factor.

The frequency of CD56 expression in our AML cases was 27.5% which was comparable to others who reported CD56 expression in 21.7% (El-Sisy et al., 2006) and 20.6% of their AML cases (Cruse et al 2005), but our results were higher than 15% reported by Chang et al. (2007) and lower than 37.8% reported by Shen et al. (2003). CD56+cases were distributed among different FAB subtypes (M1, M2, M4, M5, M6) without special prevalence to specific subtype in contrast to others who reported special prevalence in M5 subtype (Wang et al., 2005; El-Sisy et al., 2006). CD56 was expressed in 4/9 M2 cases; two of them co-expressed CD19 in accordance with others who reported that co-expression of CD19 and CD56 was found in the t (8; 21) M2 cases (Hurwitz et al., 1992; Wang et al., 2005; El-Sisy et al., 2006). Co expression of CD56 and CD7 was seen in 18% within M1subtype in accordance with Cruse et al., (2005) who reported 13% and in contrast to El Sisi et al., (2006) who reported 2.9%. Poor treatment outcome was observed in 63.4% of our CD56+AML cases, although we could not reach a statistical significance regarding OS or RFS but still supporting previous results which concluded that CD56+AML seem to be a separate entity with a worse prognosis (Xu et al., 2003; Graf et al., 2005; Ossenkoppele et al., 2011) although others reported that CD56 expression does not affect CR but it still associated with shorter OS (Chang et al., 2007; Jiang et al., 2010).

CD7, was expressed in 17.5% in agreement with Zhu et al. (2002) and comparable with Jiang et al., (2010), and it is within the values recorded earlier by different researchers (Saxena et al., 1998; Shen et al., 2003; El-Sisy et al., 2006), in contrast to higher results 15/40 reported previously (Macedo et al., 1995; Ogata et al., 2001; Cruse et al., 2005). In this work, CD7 was mostly confined to FAB M1(6/7 cases) and M2(1/7 cases) and was associated with the immature antigens CD34 in agreement with others (Saxena e al., 1998; Cruse et al., 2005; El-Sisy et al., 2006), these results support that CD7+AML originates from early hematopoietic precursors. In our cases, there was a statistical significant relation between CD7aberrant expression and unfavorable cytogenetic pattern (p=0.046), in agreement with Ogata et al. (2001) who found that the proportion of CD7+cases increased stepwise from the cases with favorable cytogenetics to the cases with intermediate and unfavorable cytogenetics, we support their recommendation that CD7 expression in AML should be interpreted in association with the cytogenetic. No statistical significance could be detected between CD7+AML and CD7- AML regarding CR or OS (p=0.68, 0.07 respectively) in agreement with Chang et al, (2007) but in contrast with Venditti et al., (1998) who reported that CD7 expression in AML adversely affect achievement of CR (p=0.013) and associated with shorter OS (p=0.006). This contradiction in the prognostic value of CD7in AML may be related to the fact that the cytogenetics status of the study population significantly skews the results where CD7 expression had no prognostic value if most examined cases had intermediate cytogenetics, while CD7+cases with unfavorable cytogenetics had an extremely poor prognosis (Ogata et al., 2001).

CD19 was expressed in 10% in accordance with others who reported 11.8% (El-Sisy et al., 2006), 8.7% (Jiang et al., 2010) and 8.6% (Bahia et al., 2001). Regarding the cytogenetic pattern of CD19+AML, it was associated with t(8;21) in 3/4 cases all of them were M2, in agreement with Bahia et al., (2001) who reported three CD19 positive cases AML-M2, and having t(8;21). The 4th case was M5 with normal karyotype [46 XY] in accordance with a single case diagnosed by Wang et al. (2009) as CD19-positive AML-M5. CD9 was expressed in 75% that was distributed among different FAB subtypes, this high prevalence of CD9 in our AML cases may be deceiving as it was done on only 8 cases, other researchers reported 29.4%,that was distributed among different FAB subtypes too (El-Sisy et al., 2006). CD22 was expressed in 7.5% in agreement with Jiang et al., (2010) who reported 8% and in contrast to the low percentage reported earlier 2.9% (El-Sisy et al., 2006).

Excluding the monocytic leukemia (M4 and M5), CD4 was expressed in 12.5% in comparison to 8.8%, one case for each M2, M3 and M5 (El-Sisy et al., 2006).

CD2 was expressed in 5% cases; M1 and M3 in agreement with Jiang et al. (2010) who reported 4.9% cases of AML expressed CD2, while in contrast to 23% reported by Lin et al, (2004).

The frequency of aberrant myeloid antigen expression in the studied ALL group was 36.4%, our results are in accordance to 39% reported by Bhushan et al. (2010), comparable to 21.2% (Paietta et al., 2001), and 55% reported by Wu et al. (2007), while in contrast to others references (Pui et al., 1998; Vitaleet al., 2007).

CD33 and CD13 were the most common antigen expressed 21.2% and 15.1% respectively, while CD15 in 6%, in contrast to others who demonstrated that CD13 (54.5%) was the most frequently expressed antigen followed by CD33 (43.0%) and CD15 (36.0%) (Seegmiller et al., 2009). Higher frequencies of CD13 (95.5%) and CD33 (81.8%) expression were reported by Wu et al. (2007) but none of their cases expressed CD15.

In the current study 9.1% cases has triple antigen expression, in accordance with Seegmiller et al. (2009) who found 19% of adult and children B-ALL have 3 or more myeloid antigen however, according to their study 25 of them met the criteria for mixed phenotypic leukemia, so the actual percentage was 6.5%, while Wiersma et al. (1991), demonstrated a lower percentage; 3.7% of patients had leukemic cells that expressed three myeloid surface antigens.

T cell -antigens (CD2, 56) were expressed in 12.1% of

the studied B-ALL cases comparable to 9% cases reported by Seegmiller et al. (2009) but they stated CD4 as the most commonly expressed T-cell antigen (nine cases) although not T- lineage specific, followed by CD2, CD5 and CD7 at lower frequency. Several smaller case series and case reports have demonstrated CD2, CD5, CD7 expression in B-ALL (Paietta et al., 2001; Peterson et al., 2007; Ahmed et al., 2008).

Up to our knowledge, we are the first to find a statistically significant higher bone marrow blast % at presentation and day 14 with p value (0.005, 0.046) respectively in the ALL cases that expressed aberrant antigens, which could be considered as high risk factors. In contrast to others who stated that no differences were found between the MyAg+and MyAg–groups with regards to bone marrow blast percentage (Vitale et al., 2007). There were no significant difference observed with regards to CR, OS, and RFS due to more intensive treatment modalities, these observations are in line with the data reported by different studies that did not detect prognostic significance of My+ALL (Jiang et al., 2010) in contrast to earlier study that report a significantly worse prognosis for My+ALL than those without (Wiersma et al., 1991).

In our study aberrant antigens were detected in less mature progenitor cells of precursor T-ALL (CD7+, CD1 -), in agreement with an earlier study that demonstrated early immature T- phenotype with aberrant expression of CD13 and CD33 (Wiersma et al., 1991). We couldnot found a statistical significace between aberrant antigen expression and early maturation antigens (CD34, CD117) in agreement with other workers (Vitale et al., 2007; Bhushan et al., 2010) and in contrast to Wu et al, (2007) who found My+ALL have higher CD34 expression rate than My-ALL.

In this work, CD56 was detected in 9% in ALL, mildly lower frequency of CD56 expression were reported earlier; 5% (Seegmiller et al., 2009), 3% (Paietta et al., 2001) and 2.2% (Hussein et al., 2011). We reported 4 cases with hyperdiploidy all were expressing CD34 and two of them lost CD45 in agreement with others who found higher CD34 expression in B-ALL with hyperdipolidy and under expression or loss of CD45 (Behm et al., 1992; Seegmiller et al., 2009).

In conclusion, our data was in agreement with previous studies, moreover we found significant higher TLC in Ly+AML than in Ly-AML and significant higher blast count in ALL cases with aberrant antigens at presentation and day 14 (p=0.005, 0.046) respectively. Shorter median RFS was noticed in AML cases that expressed ≥2 Ly antigens. No specific cytogenetic anomaly was detected for cases that express aberrant antigens but individual antigens may be related to particular cytogenetic pattern. The demonstrated immunophenotype-karyotype correlations need larger study to get benefit of these data in clinical practice either diagnostic to detect MRD or therapeutic by the use of anti-monoclonal antibodies.

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