Expression of aberrant markers in acute leukemia at South Egypt Cancer Institute: A retrospective study

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Abstract

Background*:* Aberrant phenotypes in acute leukemia (AL) have variable frequency and their prognostic relevance is controversial and dissimilar results have been reported by different groups.

Objectives*:* To determine frequency of aberrant marker expression in acute leukemia at South Egypt Cancer Institute (SECI) and to relate these expression with patient outcome and cytogenetic abnormality.

Patients and Methods: A retrospective study was conducted at SECI over a period of ten years. Total 1134 AL patients of both genders and all age groups were included in the study, of them 458 were diagnosed as acute myeloid leukemia (AML), 494 were diagnosed as B-acute lymphoblastic leukemia (B-ALL), 157 were diagnosed as T-ALL and 25 were diagnosed as biphenotypic AL (BAL).

Result*s:* Aberrant phenotypes were found in 91 (19.9%) cases of AML, 148 (30%) cases of B-ALL and 63 (40.1%) cases of T-ALL. CD56 was the most frequent aberrant marker in AML cases, CD33 in B-ALL cases and CD10 in T-ALL cases. Aberrant expression carried no significant prognostic value in AL when compared to those without aberrant expression except for that in B-ALL was a significant poor prognostic factor for relapse. CD33 was the most frequent aberrant marker in BCR/ABL positive B-ALL patients with aberrant expression, CD33 in the only t(1;19) positive B-ALL case and also the aberrantly expressed in the only t(8;14) positive B-ALL case, CD56 in PML/RARA positive AML patients and CD19 was aberrantly expressed in the two AML cases with t (8;21) and also the aberrant expressed marker in the only AML case with Monosomy 7.

Conclusion: We conclude that aberrant phenotypes were present with a considerable frequency among AL patients at SECI and may be of poor prognosis only in B-ALL. Immunophenotyping may be related to particular cytogenetic patterns. But need larger studies to confirm.

Keywords:
Acute leukemia, flow cytometry, aberrant phenotype, CD marker, prognosis, cytogenetics.

Introduction:

ALs are heterogeneous group of malignancies with varying clinical, morphologic, immunologic and molecular characteristics (1). Leukemias comprise approximately 8% of the entire human cancers, and around 50% of these cases are classified as AL (2). Leukemia is diagnosed by morphological and cytochemical examination of blast cells. Immunophenotyping, cytogenetic and molecular genetics further help in confirmation (3).

Flow cytometry (FCM) immunophenotyping plays an important role in the diagnosis and classification of AL. It also provides prognostic as well as predictive information aiding in modulating therapy appropriately (4). The morphologically similar blast cells can be easily differentiated by immunophenotyping on the basis of expression of different CD markers (5). FCM immunphentyping provide correct identification of AML−M0 (MPO cytochemistry negative AML) (6). It also identifies AML-M7 which is positive for platelet markers (7). In addition, immunophenotyping may reflect biological characteristics that the FAB and WHO classification systems do not discuss as a double phenotype and the stage of ALL These biological characteristics may affect the management and treatment protocol in patients with either AML or ALL (8).

One of the important advantage of FCM immunophenotyping is that its ability to analyze a large number of cells which improves the accuracy of leukemia diagnosis (9, 10) and this analysis can be completed within hours and is often sufficient (5).

Aberrant phenotypes in AL are defined as patterns of antigen expression on neoplastic cells different from the process of normal hematopoietic maturation due to their abnormal genetic program (11). Aberrant expression helps in sub-classifying the type of leukemia and in MRD (12). It also has variable frequency and dissimilar results have been reported by different groups (5, 13).

Some studies reported that there was no significant prognostic difference between lymphoid– (Ly-) AML and Ly+AML cases regarding OS or DFS and CR (13-15), while others noted a significantly worse prognosis for Ly+AML than those without (16, 17). Moreover, no consensus has been reached about the clinical

significance of this phenomenon in ALL. Some have demonstrated no difference in achieving CR, OS or DFS on comparing the ALL group with aberrant antigen expression with the group without (13). While others noted that the expression of myeloid antigens (MyAgs) as CD13 and CD33 has a favorable outcome in T-ALL but not in B-ALL (18), in addition others reported that T-ALL with MyAg expression was associated with unfavorable outcomes (19, 20).

FCM may predict few genetic aberrations which can be confirmed on FISH and PCR (21). The presence of multiple translocations is also associated with a higher or lower level of aberrant markers that could be effective in changing the prognosis of patients (22). No specific cytogenetic anomaly was detected for cases that express aberrant antigens but individual antigens may be related to particular cytogenetic pattern (13). Aberrant phenotype expression due to genetic defects may be associated with unfavorable outcome (12).

This study aims to determine frequency of aberrant marker expression in AL at SECI and to relate these expression with patient outcome and cytogenetic abnormality.

Patients and Methods:

This retrospective study was conducted at SECI over a period of ten years (2008-2018). With reviewing medical records of patients admitted to SECI in this period, 1134 patients of both genders and all age groups were diagnosed as AL and included in the study. FCM of the samples was performed on the Becton Dickinson (BD) fluorescence activated cell sorter (FACS) Calibur according to standard procedures.

The analysis of CD45 expression combined with side scatter was used for gating strategy. AL panel used in our laboratory includes antibodies with the following antigens: CD34, HLA-DR, CD45, myeloid panel (cytoMPO, CD13, CD33, CD14, CD117, CD36, CD64, CD41, CD61, CD11c, Antiglycophorin A), T cell panel (CD2, surface and cyto CD3, CD5, CD7, CD4, CD8, CD1a), B cell panel (CD10, CD19, CD20, CD22, cyto CD79a, Surface IgM, cyto μ, kappa and lambda).

Cases of AL were typed as conventional myeloid, B cell or T lineage, biphenotypic acute leukemia (BAL) (23). Cases with blasts co-expressing unexpected lineage associated markers are designated to have an aberrant immunophenotype (24). The frequency of aberrant expression in each group (AML, B-ALL and T-ALL) was analyzed and reported.

Follow up data was avaliable for only 277 AL patients.The patients with and without aberrant expression in each group were followed up and compared for CR achievement and occurence of relapse after CR and for the duration of DFS. Remission status was based on morphologic assessment of bone marrow aspirate (BMA) specimens at day 28 of induction therapy. CR was defined as the presence of < 5% of blasts in the BMA one month after the induction therapy was initiated (+28 days), along with the absence of blasts in peripheral blood, no extramedullary leukemia infiltrations, an absolute neutrophil count ≥ $1x10^9$/l, and platelet counts ≥ 100 x 10^9/l. Relapse was defined as a reappearance of blasts in BMA (>5%) in patients with previously documented CR. DFS is the duration from CR to the occurence of relapse (25-27).

Cytogenetic data was available for 52 patients out of 302 AL patients with aberrant expression. Analysis of the relation between aberrant expression in AL and the prescence of cytogenetic abnormality was done for occurence of CR, relapse and DFS.

As for the Statistical Analysis; Data analysis was undertaken using SPSS version 26. Categorical data were presented in form of frequencies and percentages while median and range were used to express numerical data. After testing data normality, non-parametric tests were performed. Chi square test was used to compare proportion between two different groups. Survival analysis was used and the disease-free survival time in month was assessed via a Kaplan–Meier curve, the event of interest, was the occurrence of relapse. The level of significance was considered at P value < 0.05.

Results:

A total number of patients with different types of malignancies admitted to SECI in the period from (2008- 2018) was 27551. Out of those, there were 1134 diagnosed as AL (4.11%). Age and sex distribution of AL patients illustrated in (Table 1)

As regarding types of AL; 651 (57.4%) had ALL (43.6% had B-ALL while 13.8% had T-ALL), 458 (40.4%) had AML, and 25 (2.2%) had BAL. Early T-cell precursor (ETP) represent 12.7 % from T-ALL patients.

Aberrant expression in AL

Aberrant expression in AML patients

Aberrant expression was detected in 91/458 (19.9%) AML patients. Distribution of aberrant marker expression among the patients is presented in Table 2.

Aberrant expression in B-ALL patients

Aberrant expression was detected in 148/494 (30%) B-ALL patients. Frequency of aberrant marker expression between the patients is presented in Table 3.

Aberrant expression in T-ALL patients

Aberrant expression was detected in 63/157 (40.1%) T-ALL patients including those with ETP-ALL. Frequency of aberrant marker expression among the patients is presented in Table 4.

Clinical significance of aberrant expression

From the total of 1134 AL patients, only 277 patients had complete data about their CR, relapse and DFS, and they were distributed as follow: 97 (35%) patients with AML, 143 (51.6%) with B-ALL, 33 (11.9 %) with T-ALL and 4 (1.4%) patients with BAL.

Clinical significance of aberrant expression in AML

CR was achieved in 62.5% AML patients with aberrant expression compared to 65.8% of AML patients without aberrant expression but it was

insignificant (P value=0.77). Relapse occurred more in AML patients with aberrant expression (37.5%) compared to 23.3% of AML patients without aberrant expression but the result was insignificant (P value=0.17). The median DFS in AML patients with aberrant expression was insignificantly shorter than AML patients without aberrant expression (6 months compared to 8 months) (p value=0.78) (Figure 1,2).

Clinical significance of aberrant expression in B-ALL

CR was insignificantly higher in B-ALL patients without aberrant expression compared to B-ALL patients with aberrant expression (88.3% Vs 79.6% respectively) (P value=0.16). Relapse occurred more in B-ALL patients with aberrant expression compared to those without aberrant expression (30.6% compared to 16% respectively) and it was significant (p value=0.04). The median DFS was insignificantly shorter in B-ALL patients with aberrant compared to those without aberrant expression (6 months Vs 9 months, respectively) (p value=0.86) (Figure 3,4).

Clinical significance of aberrant expression in T-ALL

CR was insignificantly higher in T-ALL patients without aberrant expression compared to those with aberrant expression (86.7% Vs 77.8% respectively) (P value=0.51). Relapse occurred in one T-ALL case with aberrant expression (5.6%) compared to two T-ALL cases without aberrant expression (13.3%) and it was insignificant (p value=0.43). The median DFS was insignificantly shorter in T-ALL patients with aberrant expression compared to those without aberrant expression (1 month compared to 7.5 months, respectively) (P value=0.66) (Figure 5).

Characteristics of cytogenetic results in AL patients with aberrant expression

Cytogenetic data was available for only 52 out of 302 AL patients with aberrant expression.

BCR/ABL (t (9;22))

It was found that there were 16 B-ALL patients with aberrant expression had negative BCR/ABL and 21 B-ALL patients with aberrant expression had positive BCR/ABL. Regarding types of aberrant markers expressed in BCR/ABL+ group; CD33 was expressed in 17 patients (81%), CD13 was expressed in 5 patients (23.8%), CD117 was expressed only in 2 patients (9.5%). While in BCR/ABL- group; CD33 was expressed in 11 patients (68.8%), CD117 was expressed in 3 patients (18.8%), CD56 was expressed in 2 patients (12.5%) and each of CD13, CD5 was expressed only in 1 patients (6.3%) (Table 5).

No statistically significant difference in occurrence of CR between negative and positive BCR/ABL patients (68.8% Vs 71.4%, respectively) (P value =0.7). Relapse was insignificantly more in BCR/ABL- compared to BCR/ABL+ group (31.3% and 23.8%, respectively) (P value=0.61). The median DFS was significantly longer in BCR/ABL+ group compared to BCR/ABL- group (11months Vs 3months respectively) (P value=0.01) (Figure 6,7).

PML/RARA (t (15;17))

It was found that there was 1 AML patient with aberrant expression had negative PML/RARA and 3 AML patients with aberrant expression had positive PML/RARA. CD56 was the only aberrantly expressed marker in either positive or negative PML/RARA patients.

t (8;21)

It was found that there were 2 AML patients with aberrant expression had positive t (8;21). Both cases expressed CD19 while CD56 was expressed in one patient.

t (1;19)

It was found that there were 2 B-ALL patients with aberrant expression had negative t (1;19) and 1 patient had positive t (1;19). CD33 was expressed in the t (1;19) positive patient and in one t (1;19) negative patient and CD117 was expressed in the other.

MLL gene re-arrangement

It was found that there were 4 AML patients with aberrant expression had negative MLL gene rearrangement. CD56 was expressed in three patients and CD19 was expressed in two patients.

t (8;14)

t (8;14) was positive in one B-All patient with aberrant expression of CD33.

Monosomy 7

Monosomy 7 was found positive in one AML patient with aberrant expression of CD19.

Table 1: Age and sex distibution of patients with acute leukemia admitted to SECI from the period of 2008-2018

Variable	Frequency N=1134	%
Age in years		
Pediatrics (< 18 years)	674	59.4
Adults (≥ 18 years)	460	40.6
Median (range)	12.00 (0.25-78.00)	
Gender		
Males	641	56.5
Females	493	43.5

Data expressed as frequency and percentage, median (range).

Table 2: Aberrant expression in patients with AML

Variable	Frequency N=458	%
Aberrant expression		
With aberrant expression	91*	19.9
Without aberrant expression	367	80.1
Aberrant marker (n=91)		
CD56	68	74.7
CD19	33	36.3
CD5	4	4.4
CD79a	1	1.1

Data expressed as frequency and percentage, median (range).
*There were some patients with more than one marker.

Table 3: Aberrant expression in patients with B-ALL

Variable	Frequency N=494	%
Aberrant expression		
With aberrant expression	148*	30.0
Without aberrant expression	346	70.0
Aberrant marker (n48=1)		
CD33	114	77.0
CD13	24	16.2
CD117	10	6.8
CD56	7	4.7
CD5	5	3.4
CD4	2	1.4
CD7	1	0.7

Data expressed as frequency and percentage, median (range).
* There were some patients with more than one marker

Table 4: Aberrant expression in patients with T-ALL

Variable	Frequency N=157	%
Aberrant expression		
With aberrant expression	63*	40.1
Without aberrant expression	94	59.9
Aberrant marker (n48=1)		
CD10	45	71.4
CD33	8	12.7
CD56	7	11.1
CD117	6	9.5
CD19	3	4.8
CD13	2	3.2

Data expressed as frequency and percentage, median (range).
*There were some patients with more than one marker

Table 5: Characteristics of B-ALL patients with aberrant expression based on BCR/ABL (t (9;22))

Variable	B-ALL with aberrant expression (n=49)		P-value*
	Negative BCR/ABL (t (9;22))	Positive BCR/ABL (t (9;22))	
Number	16*	21*	----------
Complete remission			
Yes	11 (68.8)	15 (71.4)	0.70
No	5 (31.2)	6 (28.6)	
Relapse			
Yes	5 (31.3)	5 (23.8)	0.61
No	11 (68.8)	16 (76.2)	
Median disease-free survival in months	3.00 (1.3-6.0)	11.00 (4.0-42.0)	**0.01**
Types of aberrant marker			
CD33	11 (68.8)	17 (81.0)	
CD13	1 (6.3)	5 (23.8)	
CD117	3 (18.8)	2 (9.5)	
CD56	2 (12.5)	------	
CD5	1 (6.3)	------	

Chi square test, survival analysis (Log rank test), P value is significant if < 0.05.
* There were some patients with more than one marker.

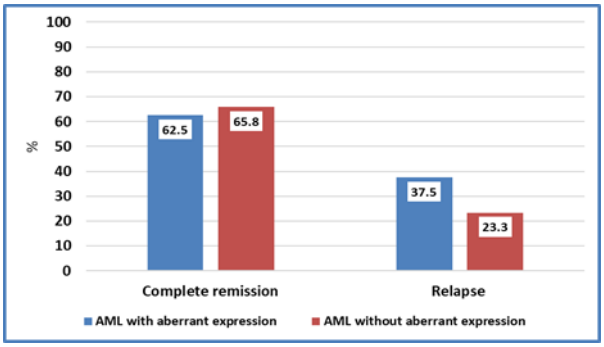

Figure 1: Complete remission and relapse in patients with AML according to their aberrant expression

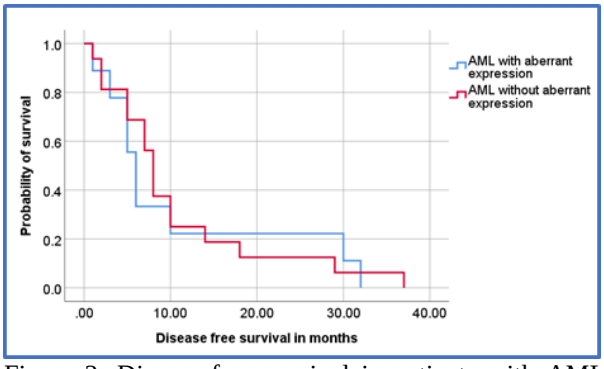

Figure 2: Disease free survival in patients with AML according to their aberrant expression

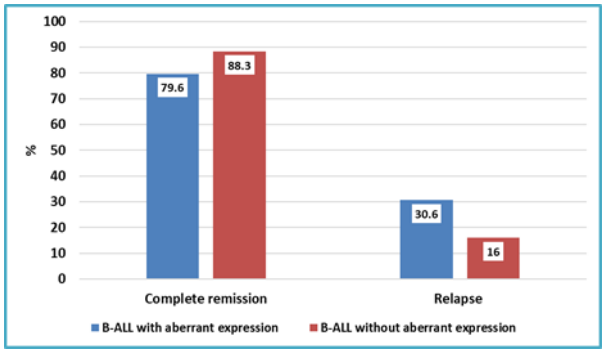

Figure 3: Complete remission and relapse in patients with B-ALL according to their aberrant expression

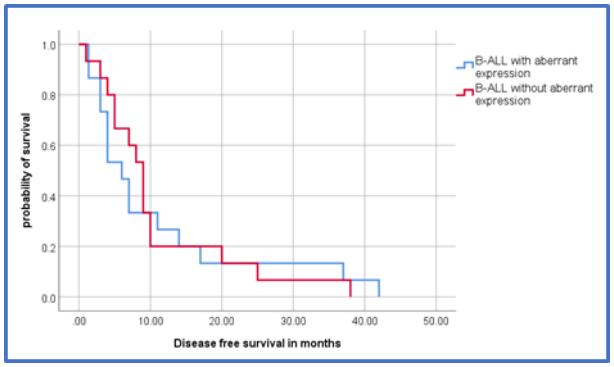

Figure 4: Disease free survival in patients with B-ALL according to their aberrant expression

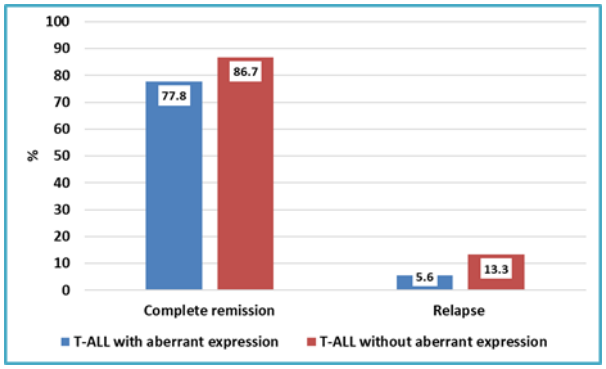

Figure 5: Complete remission and relapse in patients with T-ALL according to their aberrant expression

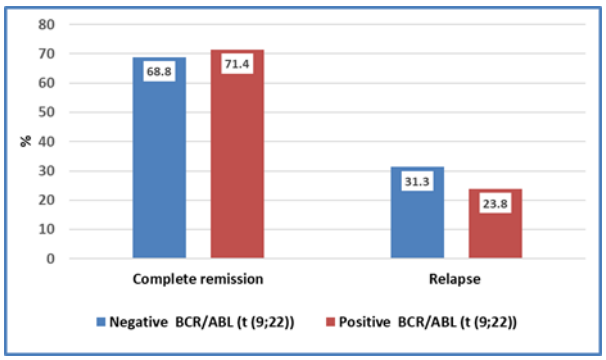

Figure 6: Complete remission and relapse in patients with patients with B-ALL based on BCR/ABL

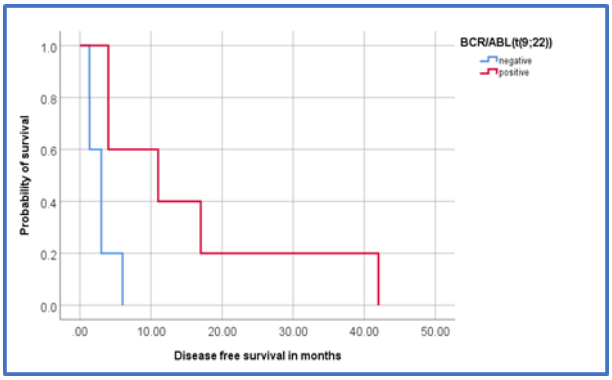

Figure 7: Disease free survival in patients with B-ALL based on BCR/ABL

Discussion:

FCM immunophenotyping is the backbone of WHO classification and plays the most crucial role in the diagnosis, lineage characterization and sub-classification of AL (4, 28). FCM is also of paramount importance in disease monitoring and prognosis through minimal residual disease (MRD) testing (29).

Aberrant expression helps in sub-classifying the type of leukemia in addition to MRD detection in leukemic patients. The detection of aberrancies by FCM is of diagnostic and prognostic importance and also helps in the decision of a tailored treatment regimen and thus the eventual outcome (12). Aberrant phenotypes in AL have variable frequency. Their prognostic relevance is controversial and dissimilar results have been reported by different groups (30).

This retrospective study was held in SECI over 10 years duration (2008- 2018). We aimed to assess the aberrant marker expression in patients newly diagnosed as AL by FCM and its effect on CR, DFS and occurence of relapse.

There was a male predominance in our AL patients, which is in accordance with most studies that noted that the males are more affected (27, 31-34). In our study there was a pediatric predominance in AL, this may be due to that ALL was more common than AML. This agrees with the results of some studies (16, 35). To the contrary of these finding, others noted that the frequency of AL was higher in adults than pediatrics (36-38).

Some studies agree with the present study and noted that ALL was more common than AML followed by BAL (5, 39-41). To the contrary of ours, some studies noted that AML was more common than ALL (16, 32, 33, 42). From our T-ALL patients, ETP-ALL represented 12.7%, which agrees with the results of many studies in which ETP-ALL ranged from 9.7% to 20% of their T-ALL cases (43-47). But a different study in adult Chinese T-ALL patients by Zhang et al., (2020), ETP-ALL was found in 47.3% (48). This variation in frequency may be due to difference in sample size, ethinity and age groups.

Aberrant expression in AML patients

The frequency of aberrant expression in AML in our study was 19.9% and this is in accordance to the results of some studies in which the aberrant expression ranged from 11.1% to 26.4% of AML cases (1, 16, 27, 36, 49). Many other studies reported higher frequency of aberrant expression among their AML patients between 30% - 67.5% (13, 18, 30, 41, 50-55). This variation in frequency of aberrant phenotypes in AML among different studies may be depending upon the markers studied, sample size or criteria of aberrancy used.

CD56 was the most common aberrant marker in our AML cases with aberrant expression (74.7%) followed by CD19 (36.3%), while CD5 and CD79a were present at lower frequency. This agrees with Abdulateef et al., (2014) who noted that CD56 was the most frequent aberrant marker in AML with aberrant expression (40.7%) followed by CD7 (25.9%), CD4 (18.5%), CD19 (14.8%), then CD2 and CD5 was expressed in 7.4% and 3.7% AML with aberrant expression, respectively (13). In a similar study in Egypt, CD56 (38%) was more common than CD19 (34%) (56).

To the contrary of ours, other studies noted different distribution of the aberrant marker expression, many of them reported that CD7 was the most common lymphoid associated antigen in AML followed by CD19 (16, 27, 32, 33, 36, 41, 57, 58). Others noted that CD19 was the most common expressed lymphoid antigen followed by CD7 (18, 53). While Azad et al., (2018) noted that CD5 and CD79a were the most common aberrant marker in AML with aberrant expression (32% for each) followed by CD7 (25%), CD10 (11%) then CD19 and CD22 (4% for each) (55). This different distribution of the aberrant antigen expression could be due to change between phenotypic characteristics of blast cells of children and adult patients and geographical change in leukemia subtypes.

Clinical significance of aberrant expression in AML

Many studies agree with ours in that aberrant expression in AML carried no significant prognostic value regarding CR when compared to those without aberrant expression (18, 50, 53, 56, 57, 59-62). Juncà et al., (2014) is also similar to ours in that CD56 expression in AML had no significant prognostic impact on DFS (60).

In contrary to current study, an observation of a meta-analysis by Xu et al., (2015) who reviewed 729 reports and 32 studies with 4074 AML patients from China, Europe, Canada, Japan, USA, Brazil, Saudi Arabia and Korea, and noted that CD56 overexpression in AML patients was associated with significant decreased in CR rate, increased relapse rate and shorter DFS (63). Also, Momani et al., (2016) noted that the aberrant expression in Jordian AML patients was associated with significantly lower CR rate and higher frequency of relapse (16). Similarly others noted that the median DFS was significantly shorter in Ly+ than Ly-AML groups either for CD56 or CD19 (56, 59). The difference in this association between aberrant expression and occurence of CR or relapse and the duration of DFS between different studies could be attributed to different treatment protocols among different countries, different follow up care of the patients during chemotherapy treatment, or different patient compliance.

Aberrant expression in B-ALL patients

In our study 30% of B-ALL patients had aberrant expression. This comes in agreement with few studies. Momani et al., (2016) noted that 29% of B-ALL cases had aberrant expression (16). Also Chughtai et al., (2013) reported that 25% of B-ALL cases had aberrant phenotypes (50). Our finding was higher than the study of Khurram et al., (2010) in which the proportional frequency of aberrant expresson in 55 B-ALL caes was 10.9% (41). While our study result was lower than a study carried out in Sohag by Abdullah et al., (2018) who noted that the aberrancy was seen in 46% out of 15 B-ALL cases (32). Also Sarma et al., (2015b) noted that 59.2% cases of B-ALL had aberrant phenotypes (30).

Many studies agree with Ours and noted that aberrant MyAg expression in B-ALL was more common than T-cell antigen. Ohki et al., (2020) noted that the aberrant MyAg expression in B-ALL (in the form of CD13, CD15, CD33, and CD65, CD117) was more common than T-cell antigen and CD56 expression (46). Momani et al., (2016) held a study in Jordan and noted that CD33 was the commonest aberrant marker in B-ALL patients with aberrant expression (60.5%) followed by CD13 (50%) while CD7 was expressed in only one case (3%) (16), this figure was also present in another study by Alkayed et al., (2015) on Jordanian children with B-ALL (64). Many other studies reported that CD33 was more common than CD13 (36, 65-67).

Other studies were similar to ours in that aberrant expression of MyAg in B-ALL was more common than T-cell antigens but with different distribution of myeloid markers where CD13 was the most common aberrant marker followed by CD33 (21, 68). The same was noted byAbdullah et al., (2018) and Chughtai et al., (2013), but there was no aberrant T-cell antigen in their B-ALL cases (32, 50).

Clinical significance of aberrant expression in B-ALL

Many studies agree with the current study in that there were no significant difference in CR between the MyAg+and MyAg– ALL groups (13, 14, 18, 69-71). Yenerel et al., (2002) and Antonella et al., (2007) are similar to ours in that there was no difference in DFS between the MyAg+and MyAg– ALL groups (69, 70).

In contrast to ours, Momani et al., (2016) found that the aberrant MyAg expression in ALL had significant adverse effect on CR achievement (16). Others noted that the aberrant MyAg in B-ALL did not affect the relapse rate (70, 71). The difference between the studies could be difference in treatment protocols and follow up care among differnt countries which may affect the CR achievement, occurence of relapse after CR or DFS.

Aberrant expression in patients with T-ALL

In the present study 40.1% of T-ALL patients had aberrant expression including those with ETP-ALL.

This was in accordance with Al-Saadi et al., (2020) who noted that 44.5% out of 45 T-ALL cases had aberrant phenotyes (72). Also Momani et al., (2016) found that out of 42 T-ALL cases, 28.6% showed aberrant antigen expression (16). Similarly Khurram et al., (2010) noted that the proportional frequency of aberrant expression in 18 T-ALL cases was 27.77% (41). But Narang et al., (2017) reported much lower frequency (9.1%) than ours (36). While Chughtai et al., (2013) and Sarma et al., (2015b) noted a higher frequency of aberrant expression (57% and 66.7% of T-ALL cases, respectively) (30, 50).

Many studies agree with ours in that aberrant expression of B-cell marker in T-ALL was more common than aberrant myeloid marker and also CD10 was the most common aberrant marker (1, 19, 73). Garg et al., (2018) is similar to the current study in that aberrant expression of B-cell Ag in Indian T-ALL patients was more common than aberrant MyAg but CD79a was the most frequent (47.36% out of 40 T-ALL) followed by CD117 (42.28% out of 40 T-ALL), CD33 (38.46% out of 40 T-ALL) and lastly CD10 (35.3% out of 40 T-ALL) (74).

In contrast to ours, other studies noted that aberrant expression of MyAg in T-ALL was more common than B-cell Ag (16, 27, 50, 74). While others reported that there was no aberrant B-cell Ag in T-ALL cases (41, 66). The variation in the frequency and distribution of aberrant markers in ALL may be due to variations in age, gender distribution and/or ethinity. In addition, there is variation in the number of samples and number of monoclonal antibodies (MoAbs) used which varies from only 2 MoAbs to as many as 6.

Clinical significance of aberrant expression in T-ALL

Tong et al., (2014) agree with ours in that the presence of aberrant MyAg did not affect the cumulative incidence of relapse and CR achievement in adult Chinese T-ALL patients (71). Similarly Antonella et al., (2007) found that there were no differences in CR achievement and DFS between MyAg+ and MyAg- adult T-ALL cases (69).

To contrary of the present study, Supriyadi et al., (2012) noted that aberrant MyAg expression in T-ALL was a significant adverse prognostic factor where My+T-ALL patients had a higher chance of having induction failure and 2 out of 10 patients failed induction while no patient with My-T-ALL (48 patients) had induction failure (19). The difference in these results may be attributed to variation in sample size, mangement, supportive care and other factors as early diagnosis and compliance of patients to therapy.

Relation of aberrant expression with Cytogenetics in AL patients

Jaso et al., (2011) agree with ours and noted that that BCR/ABL+ adult B-ALL patients had a greater frequency of CD13 (75). Corrente et al., (2018) also agree with the present study and noted that BCR/ABL+ cases had a greater frequency of CD13 and CD33 positivity compared with BCR/ABL- adult B-ALL cases (76). Similarly Azam et al., (2020) found that CD13 was significantly higher BCR/ABL+ compared with BCR/ABL- ALL cases (77). In contrast to ours, Abdulateef et al., (2014) who noted that none of the 3 BCR/ABL+ALL cases expressed MyAg and only one of them expressed CD56 (13).

Antonella et al., (2007) agree with ours in that there was no difference in CR achievement between MyAg+ and MyAg- BCR/ABL+B-ALL cases, but was in contrary to ours in that DFS did not differ between MyAg and MyAg cases in BCR/ABL+B-ALL (69). Similarly Craddock et al., (2013) noted that aberrant expression of CD13 was a significant adverse prognostic factor for survival in adults with BCR/ABL negative B-ALL (78).

CD56 was the only aberrantly expressed marker in AML patients with aberrant expression either positive or negative PML/RARA, supporting the results of (Chen et al., 2008) who found that CD56 was the only aberrant marker and was present in one (17%) out of 6 AML-M3 with t (15;17) (61). In the contrast to the present study, other studies found that aberrant Ly Ag was not detected in t (15;17) positive AML patients (79, 80). While Shorbagy et al., (2016) noted that among 6 AML-M3 cases with t (15;17), no case expressed CD56 and only one case had CD19+ (56).

CD19 was expressed in the two patients and CD56 expressed in one patient. This agrees with many studies which noted that CD19 and CD56 were present with high frequency in AML cases with t (8;21) (61, 79, 80). Also Abdulateef et al., (2014) noted that CD19+AML was associated with t(8;21) in 3/4 AML patients (13). Similarly Pardo et al., (2020) found that CD56 expression was an important identifying phenotypic feature in pediatric AML patients with t (8;21) (81).

In conclusion, our results showed that the aberrant phenotypes were presented in a considerable frequency in patients with various subtypes of AL at SECI. The frequency of aberrant phenotype was higher in T-ALL than in B-ALL followed by AML. CD56 was the most commonly expressed lymphoid antigen followed by CD19 in AML. CD33 was the most commonly expressed aberrant antigen followed by CD13 in B-ALL. CD10 was the most commonly expressed aberrant antigen followed by CD33 in T-ALL. Aberrant antigen expresion in AL subtypes was of no prognostic significance as regard CR achievement, DFS duration and occurence of relapse after remission except in B-ALL where it was a poor prognostic factor for DFS. Individual aberrant antigens may be related to particular cytogenetic patterns. Aberrant phenotypes-cytogenetics correlations need larger studies for confirmation.

Conclusion:

We conclude that aberrant phenotypes were present with a considerable frequency among AL patients at SECI and may be of poor prognosis only in B-ALL. Immunophenotyping may be related to particular cytogenetic patterns. But need larger studies to confirm.

List of Abbreviations:

AL	Acute Leukemia
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
BAL	Biphenotypic acute Leukemia
CD	Cluster of Differentiation
Cyto	Cytoplasmic
MyAg	Myeloid antigen
Ly	Lymphoid
WHO	World Health Organization
FAB	French-American-British.
MPAL	Mixed phenotype acute leukemia
ETP	Early T cell Precursor
FACS	Fluorescence Activated Cell Sorting
FCM	Flow cytometry
Mo Abs	Monoclonal antibodies
MRD	Minimal residual disease
BD	Becton Dickinson
CR	Complete remission
DFS	Disease free survival
SECI	South Egypt Cancer Institute
BMA	Bone marrow aspirate
CD	Cluster of differentiation

Competing Interests:

There are no competing interests.

Authors' Contributions:

M.M. have carried out the collection of data, analysis and interpretation of data and drafted the manuscript.

E.S. & A.A. have contributed to designing the work.

D.S. has contributed by supervising and revising the work.

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References:

1. Salem DA, Abd El-Aziz SM. Flowcytometric immunophenotypic profile of acute leukemia: mansoura experience. Indian journal of hematology and blood transfusion. 2012;28(2):89-96.
2. Ramezani M, Hosseini S, Vaisi-Raygani A, et al. Aberrant markers expression in leukemia patients: a report from Western Iran. Scholars Journal of Applied Medical Sciences. 2016;4:1035-8.
3. Angelescu S, Berbec NM, Colita A, et al. Value of multifaced approach diagnosis and classification of acute leukemias. Mædica. 2012;7(3):254.
4. Paietta E. Immunobiology of acute leukemia. Neoplastic diseases of the blood: Springer; 2018. p. 237-79.
5. Sarma A, Hazarika M, Das D, et al. Expression of aberrant CD markers in acute leukemia: A study of 100 cases with immunophenotyping by multiparameter flowcytometry. Cancer Biomarkers. 2015;15(4):501-5.
6. Arber DA, Borowitz MJ, Cessna M, et al. Initial diagnostic workup of acute leukemia: guideline from the College of American Pathologists and the American Society of Hematology. Archives of pathology & laboratory medicine. 2017;141(10):1342-93.
7. Creutzig U, van den Heuvel-Eibrink MM, Gibson B, et al. Diagnosis and management of acute myeloid leukemia in children and adolescents: recommendations from an international expert panel. Blood. 2012;120(16):3187-205.
8. Ouyang G, Xu Z, Jiang D, et al. Clinically useful flow cytometry approach to identify immunophenotype in acute leukemia. Journal of International Medical Research. 2019;47(4):1483-92.
9. Zeijlemaker W, Kelder A, Oussoren-Brockhoff Y, et al. A simple one-tube assay for immunophenotypical quantification of leukemic stem cells in acute myeloid leukemia. Leukemia. 2016;30(2):439.
10. Iriyama N, Asou N, Miyazaki Y, et al. Normal karyotype acute myeloid leukemia with the CD7+ CD15+ CD34+ HLA-DR+ immunophenotype is a clinically distinct entity with a favorable outcome. Annals of hematology. 2014;93(6):957-63.
11. Gupta M, Gupta S, Singh S, et al. Aberrant expression of cd markers in acute lymphoblastic leukemia: A diagnostic clue of malignancy or compounding confusions. Indian Journal of Scientific Research. 2017;8(1):81-5.
12. Shahni A, Saud M, Siddiqui S, et al. Expression of aberrant antigens in hematological malignancies: A single center experience. Pakistan journal of medical sciences. 2018;34(2):457.
13. Abdulateef N, Ismail MM, Aljedani H. Clinical significance of co-expression of aberrant antigens in acute leukemia: a retrospective cohort study in Makah Al Mukaramah, Saudi Arabia. Asian Pac J Cancer Prev. 2014;15(1):221-7.
14. Jiang N-G, Chen X-M, Zhu H-L, et al. Immunophenotype characteristics and prognosis of acute leukemia patients with cross expressing lymphoid and myeloid lineage associated antigens. Zhongguo shi yan xue ye xue za zhi. 2010;18(6):1405-9.
15. Aparna SK, Sharmila M. Aberrant phenotypes in acute myeloid leukemia in India. International Journal of Advances in Medicine. 2018;5(2):361.
16. Momani A, Alsokhni H, Habahbeh L, et al. Aberrant Antigen Expression in Patients with Acute Leukemias: Experience of King Hussein Medical Center in Jordan. Journal of the Royal Medical Services. 2016;102(3396):1-9.

17. Zhu H, Niu T, Meng W, et al. Immunophenotype of acute leukemia and its clinical significance. Hua xi yi ke da xue xue bao= Journal of West China University of Medical Sciences= Huaxi yike daxue xuebao. 2002;33(1):118-20.
18. Bhushan B, Chauhan PS, Saluja S, et al. Aberrant phenotypes in childhood and adult acute leukemia and its association with adverse prognostic factors and clinical outcome. Clinical and experimental medicine. 2010;10(1):33-40.
19. Supriyadi E, Veerman AJ, Purwanto I, et al. Myeloid antigen expression in childhood acute lymphoblastic leukemia and its relevance for clinical outcome in indonesian ALL-2006 protocol. Journal of oncology. 2012;2012.
20. Mugairi A, Dalal B, Pi S, et al. Thymic Immunophenotype, and Expression of CD4 and Myeloid Antigens is Associated with Outcome in Adult Patients with T–Cell Acute Lymphoblastic Leukemia. J Leuk 3: 172. doi: 10.4172/2329-6917.1000172 Page 2 of 6 J Leuk ISSN: 2329-6917 JLU, an open access journal Volume 3• Issue 1• 1000172. Asians Nine (33%) patients had splenomegaly. 2014;11(42):17-66.
21. Seegmiller AC, Kroft SH, Karandikar NJ, et al. Characterization of immunophenotypic aberrancies in 200 cases of B acute lymphoblastic leukemia. American journal of clinical pathology. 2009;132(6):940-9.
22. Kavianpour M, Ketabchi N, Saki N. Prognostic significance of aberrant expression of CD markers in acute lymphoblastic leukemia. memo - Magazine of European Medical Oncology. 2017;10(3):164-9.
23. Ludwig W, Matutes E, Orfao A, et al. Proposals for the immunological classification of acute leukemia. Leukemia. 1995;9(10):1783-6.
24. Ossenkoppele GJ, van de Loosdrecht AA, Schuurhuis GJ. Review of the relevance of aberrant antigen expression by flow cytometry in myeloid neoplasms. British journal of haematology. 2011;153(4):421-36.
25. Whitman SP, Ruppert AS, Radmacher MD, et al. FLT3 D835/I836 mutations are associated with poor disease-free survival and a distinct gene-expression signature among younger adults with de novo cytogenetically normal acute myeloid leukemia lacking FLT3 internal tandem duplications. Blood. 2008;111(3):1552-9.
26. Cui W, Zhang D, Cunningham MT, et al. Leukemia-associated aberrant immunophenotype in patients with acute myeloid leukemia: changes at refractory disease or first relapse and clinicopathological findings. International Journal of Laboratory Hematology. 2014;36(6):636-49.
27. Rodríguez-Rodríguez S, Pomerantz A, Demichelis-Gómez R, et al. Impact of aberrant antigens in the outcome of patients with acute leukemia at a referral institution in Mexico City. Revista de Investigación Clínica. 2017;68(6):305-13.
28. Gupta N, Pawar R, Banerjee S, et al. Spectrum and immunophenotypic profile of acute leukemia: a tertiary center flow cytometry experience. Mediterranean Journal of Hematology and Infectious Diseases. 2019;11(1).
29. Zhou Y, Wood BL. Methods of detection of measurable residual disease in AML. Current hematologic malignancy reports. 2017;12(6):557-67.
30. Sarma A, Hazarika M, Das D, et al. Expression of aberrant CD markers in acute leukemia: A study of 100 cases with immunophenotyping by multiparameter flowcytometry. Cancer Biomarkers. 2015;15:501-5.
31. Liu J, Tan X, Ma Y-Y, et al. Study on the Prognostic Value of Aberrant Antigen in Patients With Acute B Lymphocytic Leukemia. Clinical Lymphoma Myeloma and Leukemia. 2019;19(7):e349-e58.
32. Abdullah NF, Wafa A-E, Ahmed H, et al. Mohamed AM. Aberrant Expression of CD Markers in Cases with Acute Leukemia in Sohag University Hospital. Sohag Medical Journal. 2018;22(2):287-95.
33. Hamed EO, El-Deen AF. Flow Cytometric Diagnosis of Acute Leukemia and Aberrant Antigen: Sohag University Experience. Open Journal of Blood Diseases. 2018;8(2):37-48.
34. Eswaran Y. PREVALENCE OF LEUKEMIA IN TERTIARY CARE HOSPITALS. Indian Journal of Applied Research. 2020;9(12).
35. Shrestha S, Shrestha J, Pun C, et al. Immunophenotypic study of acute leukemia by flow cytometry at BPKMCH. Journal of pathology of Nepal. 2013;3(5):345-50.
36. Narang V, Dhiman A, Garg B, et al. Immunophenotyping in Acute leukemias: First tertiary care centre experience from Punjab. Indian Journal of Pathology and Oncology. 2017;4(2):297-300.
37. Koju S, Sachdeva MUS, Bose P, et al. Spectrum of acute leukemias diagnosed on flow cytometry: Analysis from tertiary care centre from North India. Annals of Clinical Chemistry and Laboratory Medicine. 2015;1(1):12-5.
38. Abbasi N, Kamal N, Al-Kaisi N, et al. Immunophenotypic Profile of Acute Leukemia Cases Using Multicolor Flow Cytometry: Three Year Experience at King Hussein Medical Center. Journal of the Royal Medical Services. 2015;102(2028):1-6.
39. Aparajita Das PM, Sudha Sethy, Bidyut Prava Das. Immunophenotyping in acute leukaemiaan institutional study. Journal of Evidence Based Medicine & HealthCare. 2018:600-4.
40. Kalam AA, Khan MR, Habib AH, et al. Uncommon CD markers in acute myeloid leukemia. Bangabandhu Sheikh Mujib Medical University Journal. 2018;11(4):267-9.
41. Khurram MM, Jafri SA, Mannan A. Frequency of aberrant expression of CD markers in cases of acute leukemia. Medical Journal of Islamic World Academy of Sciences. 2010;109(395):1-6.
42. Khakhlari N, Gogoi B, Barua A, et al. A Study of Aberrant Phenotypes in Acute Leukemia by

Flowcytometry. International Journal of Medical Research Professionals. 2016;2.
43. Coustan-Smith E, Mullighan CG, Onciu M, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. The lancet oncology. 2009;10(2):147-56.
44. Genescà E, Morgades M, Montesinos P, et al. Unique clinico-biological, genetic and prognostic features of adult early T-cell precursor acute lymphoblastic leukemia. haematologica. 2020;105(6):e294.
45. Jain N, Lamb AV, O'Brien S, et al. Early T-cell precursor acute lymphoblastic leukemia/lymphoma (ETP-ALL/LBL) in adolescents and adults: a high-risk subtype. Blood. 2016;127(15):1863-9.
46. Ohki K, Takahashi H, Fukushima T, et al. Impact of immunophenotypic characteristics on genetic subgrouping in childhood acute lymphoblastic leukemia: Tokyo Children's Cancer Study Group (TCCSG) study L04-16. Genes, Chromosomes and Cancer. 2020;59(10):551-61.
47. Noronha EP, Marques LVC, Andrade FG, et al. T-lymphoid/myeloid mixed phenotype acute leukemia and early T-cell precursor lymphoblastic leukemia similarities with NOTCH1 mutation as a good prognostic factor. Cancer Management and Research. 2019;11:3933.
48. Zhang Y, Qian J-J, Zhou Y-L, et al. Comparison of Early T-Cell Precursor and Non-ETP Subtypes Among 122 Chinese Adults With Acute Lymphoblastic Leukemia. Frontiers in oncology. 2020;10:1423.
49. Launder TM, Bray RA, Stempora L, et al. Lymphoid-associated antigen expression by acute myeloid leukemia. American journal of clinical pathology. 1996;106(2):185-91.
50. Chughtai O, Chughtai A. Aberrant expression of CD markers in acute leukemia. Ann Pak Inst Med Sci. 2013;9(2):99-102.
51. Jahedi M, Shamsasenjan K, Sanaat Z, et al. Aberrant phenotype in Iranian patients with acute myeloid leukemia. Advanced pharmaceutical bulletin. 2014;4(1):43.
52. Jha R, Grover G, Bose P. Lymphoid associated antigen expression in new cases of Acute Myeloid Leukemia. Journal of Pathology of Nepal. 2013;3(6):487-90.
53. Jeong SH, Lee HW, Kang SY, et al. Clinical significance of co-expression of aberrant antigens in acute leukemia. The Korean Journal of Hematology. 2009;44(2):67-73.
54. Al-Anizi WM, Al-Mashta MAR. The frequency of aberrant lymphoid antigens expression in 202 Iraqi patients with de novo acute myeloid leukemia. Iraq Joural of Hematology. 2017;6(2):49-54.
55. Azad AK, Khan MR, Habib AH, et al. Aberrant Expression of CD Markers in Acute Myeloid Leukaemia. Haematology Journal of Bangladesh. 2018;2(01):14-6.
56. Shorbagy S, Haggag R, Alazizi N, et al. CD56 and CD19 antigens expression in acute myeloid leukemia identifies patients with adverse prognosis in Egypt. Int J Science Res. 2016;5(1):2319-7064.
57. Muhsin SY, Al-Mudallal SS. Expression of Aberrant Antigens CD7 and CD19 in Adult Acute Myeloid Leukemia by Flow Cytometry. Iraq Joural of Hematology. 2014;3(1):1-13.
58. Jiang N, Chen X, Zhu H, et al. Immunophenotype characteristics and prognosis of acute leukemia patients with cross expressing lymphoid and myeloid lineage associated antigens. Zhongguo shi yan xue ye xue za zhi. 2010;18(6):1405-9.
59. Coelho-Silva JL, Carvalho LE, Oliveira MM, et al. Prognostic importance of CD56 expression in intermediate risk acute myeloid leukaemia. British Journal of Haematology. 2016;176(3):498-501.
60. Juncà J, Garcia-Caro M, Granada I, et al. Correlation of CD11b and CD56 expression in adult acute myeloid leukemia with cytogenetic risk groups and prognosis. Annals of Hematology. 2014;93(9):1483-9.
61. Chen SW, Li CF, Chuang SS, et al. Aberrant co-expression of CD19 and CD56 as surrogate markers of acute myeloid leukemias with t (8; 21) in Taiwan. International Journal of Laboratory Hematology. 2008;30(2):133-8.
62. Ng S, Ariffin W, Lin H, et al. Clinical features and treatment outcome of children with myeloid antigen coexpression in B-lineage acute lymphoblastic leukemia: a study of 151 Malaysian children. Journal of tropical pediatrics. 2000;46(2):73-8.
63. Xu S, Li X, Zhang J, et al. Prognostic value of CD56 in patients with acute myeloid leukemia: a meta-analysis. Journal of Cancer Research and Clinical Oncology. 2015;141(10):1859-70.
64. Alkayed K, Khattab E, Madanat F. Aberrant T-cell antigen expression in Jordanian children with B lymphoblastic leukemia. Hematology/oncology and stem cell therapy. 2015;8(4):187-8.
65. Wimalachandra M, Prabashika M, Dissanayake M, et al. Immunophenotypic characterization of acute lymphoblastic leukemia in a flowcytometry reference centre in Sri Lanka. Ceylon Medical Journal. 2020;65(1-2).
66. Haddad F, Wraikat A, Khasawneh R, et al. Immunophenotypic Diagnosis of Acute Lymphoblastic Leukemia Using Flow Cytometry: Experience at King Hussein Medical Center. Journal of the Royal Medical Services. 2014;102(1193):1-6.
67. Salem DA, El-Aziz SMA. Flowcytometric immunophenotypic profile of acute leukemia: mansoura experience. Indian journal of hematology and blood transfusion. 2012;28(2):89-96.
68. Sharma M, Sachdeva MUS, Varma N, Varma S, et al. Characterization of immunophenotypic aberrancies in adult and childhood acute lymphoblastic leukemia: A study from Northern India. Journal of cancer research and therapeutics. 2016;12(2):620.
69. Antonella V, Anna G, Cristina A, et al. Absence of prognostic impact of CD13 and/or CD33 antigen expression in adult acute lymphoblastic leukemia.

Results of the GIMEMA ALL 0496 trial. Haematologica. 2007;92(3):342-8.
70. Yenerel M, Atamer T, Yavuz A, et al. Myeloid antigen expression provides favorable outcome in patients with adult acute lymphoblastic leukemia: a single-center study. Annals of hematology. 2002;81(9):498-503.
71. Tong H, Wang H, Wang Q, et al. Immunophenotypic, cytogenetic and clinical features in Chinese adult acute lymphoblastic leukaemia (ALL) patients. Ann Acad Med Singapore. 2014;43(3):152-9.
72. Al-Saadi EAKD, Abdulnabi MA, Jaafar FH. Characterization of flow cytometric immuno-phenotyping of acute myeloid leukemia with minimal differentiation and acute T-cell lymphoblastic leukemia: A retrospective cross-sectional study. F1000Research. 2020;9(1170):1170.
73. Jaafar F, Kadhom A. Expression of CD45, CD34, CD10, and human leukocyte antigen-DR in acute lymphoblastic leukemia. Iraqi Journal of Hematology. 2018;7(1):14-9.
74. Garg N, Kotru M, Kumar D, et al. Correlation of expression of aberrant immunophenotypic markers in T-ALL with its morphology: A pilot study. Journal of laboratory physicians. 2018;10(4):410.
75. Jaso J, Thomas DA, Cunningham K, et al. Prognostic significance of immunophenotypic and karyotypic features of Philadelphia positive B-lymphoblastic leukemia in the era of tyrosine kinase inhibitors. Cancer. 2011;117(17):4009-17.
76. Corrente F, Bellesi S, Metafuni E, et al. Role of flow-cytometric immunophenotyping in prediction of BCR/ABL1 gene rearrangement in adult B-cell acute lymphoblastic leukemia. Cytometry Part B: Clinical Cytometry. 2018;94(3):468-76.
77. Azam S, SI, MNI, MIH, Islam KA, et al. Frequency of BCR-ABL Positive Acute Lymphoblastic Leukaemia in a Single Centre Study in Dhaka. International Journal For Research In Medical Reasearch professionals (ISSN: 2454-6364). 2020.
78. Craddock KJ, Chen Y, Brandwein JM, et al. CD13 expression is an independent adverse prognostic factor in adults with Philadelphia chromosome negative B cell acute lymphoblastic leukemia. Leukemia Research. 2013;37(7):759-64.
79. Wang X-B, Zheng J-E, Gu J-X, et al. Correlation of immunophenotype to cytogenetics and clinical features of adult acute myeloid leukemia. Ai zheng= Aizheng= Chinese journal of cancer. 2005;24(6):667-71.
80. Zheng J, Wang X, Hu Y, et al. A correlation study of immunophenotypic, cytogenetic, and clinical features of 180 AML patients in China. Cytometry Part B: Clinical Cytometry: The Journal of the International Society for Analytical Cytology. 2008;74(1):25-9.
81. Pardo LM, Voigt AP, Alonzo TA, et al. Deciphering the Significance of CD56 Expression in Pediatric Acute Myeloid Leukemia: A Report from the Children's Oncology Group. Cytometry Part B: Clinical Cytometry. 2020;98(1):52-6.