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Aberrant CD markers expression in acute leukaemia frequency and intensity and its association with other CBC parameters

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KEY WORDS:
Aberrant expression, Coagulopathy, Leukaemia Phenotype,.

ABSTRACT
Background: To enhance our knowledge concerning acute leukaemia investigation profile and try to have a useful application in disease management.
Objective: To find the associations between acute leukaemia phenotypes, a cluster of differentiation (CD) markers, and complete blood cell count .
Subjects and methods: A retrospective study was conducted in the period 2021 to 2022 in Baghdad medical city in the oncology centre, included 72 acute leukaemic newly diagnosed patients, diagnosis of the cases was based on history, physical examination, complete blood picture, bone marrow aspirate and biopsy examination, flow cytometric profile was done, all our cases were new cases without previous treatment and the flow cytometric reports were conducted before treatment.
The differences in groups for this study were evaluated by t-test, an association test were used, and a P value < 0.05 is considered significant.
Results: Cases with aberrancy have higher mean white blood cells count, which may indicate an inferior prognosis. Cases with aberrancy have higher mean platelets distribution width, there is an inverse correlation between platelets count and platelets distribution width, this may suggest in our opinion a higher susceptibility to coagulation system activation and hence disseminated intravascular coagulopathy in those patients. In acute lymphoblastic leukaemia (ALL), cytoplasmic CD79a (a B lymphocyte cell marker used in the diagnosis of B- ALL) negative cases and in acute myeloid leukaemia (AML) CD 117(a myeloblast marker in AML) negative both have higher mean absolute white blood cells count.
Conclusion: Acute leukemia with aberrant phenotypes cases is suspected to be more prone to have coagulopathy, an inferior prognosis.

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INTRODUCTION

Acute leukaemia is a prevalent disease affecting blood cell precursors in bone marrow in children and adult patients[1,2]. In order to diagnose and manage acute leukaemia, the required investigation include complete blood count (CBC), bone marrow aspirate and biopsy, cytochemical stains, flowcytometry, FISH (Fluorescence in situ hybridization) technique, and polymerase chain reaction, all these techniques are complementary to each other [3].

Some authors point to the significance of platelets count, platelets indices in hypoplatelets production and immune thrombocytopenic patients, they talk about its predictive value of these indices and the effects on disease management [4], in this study platelet count significance is also considered.

Aberrancy or aberrant expression of cluster of differentiation (CD) markers is a term in flow cytometric reports and it has a growing clinical impact on disease management, aberrancy is the expression of a line-specific marker on another lineage [5]. The association between aberrancy and CBC in collected acute leukaemic patients were inspected in this study. Here we will focus on some aspects of flow cytometric findings and their relation to some CBC findings which facilitate further understanding of the nature of acute leukaemia which has positive impacts on disease management and trying to find any predictive value, also trying to find further useful findings.

MATERIAL

A retrospective study conducted on 72 newly diagnosed acute leukaemic patients was performed in the period 2021 to 2022 over eleven months in Bagdad Medical City and College of Pharmacy/University of Mosul. Ethical approval was obtained from the Collegiate Committee for Medical Research Ethics / University of Mosul, No.: 99 on 29-12-2022.Diagnosis of the cases was based on history, clinical examination, CBC, bone marrow examination (aspirate and biopsy), flow cytometric profile using data analysis was performed through BD FACS-CantoII system, using FACS CANTO Diva software (Becton-Dickinson, UK). Blast gated using CD45 and SSC together, 10% and more were considered positive for nuclear CD marker and 20% and more were considered positive for surface CD marker, inclusion criteria were cases of newly diagnosed without previous treatment and the flow cytometric reports were conducted before treatment., bone marrow, blood samples were placed in EDTA tubes and examined within 24 hours for the flow cytometer, otherwise, the cases were excluded.

For diagnosis, the following CD markers approach was studied [6].

The initial panel is designed to detect the following CD markers cMPO, cCD79a, CD19, CD3, cCD3, CD7, CD34, CD45, and followed accordingly by one of the following panels:

For AML(Acute myeloid leukaemia): CD16, CD35, CD36, cCD TdT, CD41a, CD42a/61 , CD13, CD64, CD305, CD56, CD25, CD203, CD117, CD11b, CDIREM-2, CD33, CD7, CD42b, CD123, CD10, CD14, CD71, CD19, CD9, CD4, CD34, CD HLA DR and CD 45.

For B-Cell ALL (Acute lymphoblatic leukaemia): CD58, cCD IgM, cCD TdT,

cCD66, CD13, CD19, CD22, CD10, CD117, CD IgM, CD38, CD lambda, CD34, CD20, CD Kappa, CD9, CD45.

For T-Cell ALL: cCDTdT, CD2, CD HLA-DR, CD99, CD117, CD13, CD10, CD8,CD56, CD1a, CD7, CD3, CD5, CD4, CD33,cCD3, CD45.

Marker of aberrancy in acute leukemia [5]:

I-Aberrant antigen in AML: most commonly Including the following CD 56 , CD19, CD7.CD2 .

II-Aberrant CD markers expression in B cell – ALL:

1- The following CD markers are included CD 65 or CD33, CD 15, CD 14, CD 13 ..

2- The following CD markers are included CD 64, CD36 and CD 11b.

3- T-natural killer antigen expression in B cell -ALL, mostly CD56, and CD4.

III-Aberrant antigen expression in T cell- ALL: myeloid Ag (CD 13 & CD 33) with CD56, CD79a.

Statistical analysis :

The data analysis was conducted using SPSS 23.0 (SPSS Inc., Chicago, IL, USA) was used for data analyses. The differences in groups for this study were evaluated by t-test, an association test were used, and a P value < 0.05 is considered significant. CI equal to 95%, equal variances assumed.

RESULTS

In the present study, a total of 72 acute leukaemia patients were enrolled, 37 ALL (acute lymphoblastic leukaemia) patients (8 T-ALL, and 29 B-ALL Patients) and 35 AML(acute myeloid leukaemia) patients, with mean age (years) 32.1±26.7 (minimum age was 1 year and maximum 92 years).

There is a statistically significant difference in the mean age between 37 ALL cases (17.6 years) and 35 AML cases (47.5 years) (t-test $P<0.05$, CI=95%). Mean WBC (White blood cells) count in ALL patients (37 cases, $81.51x10^9/L$) versus AML patients (35 cases, $25.33\ x10^9/L$), means difference was statistically significant (t-test $P<0.05$).

The characterization of aberrant cases of newly diagnosed leukaemic patients has been done and the outcome is listed in (Table 1).

Table 1. Aberrancy in acute leukaemia frequency and intensity.

	Acute leukaemia type	Aberrant phenotype	% Of aberrancy and intensity
	B-ALL (29 cases)		**7% Percentage of aberrancy**
1		CD 33	Dim
2		CD13, CD 33	Heterogeneous
	T-ALL (8 cases)		**25% Percentage of aberrancy**
1		CD 33, CD 117	Moderate, heterogeneous respectively
2		CD 13	Dim
	AML (35 cases)		**29% Percentage of aberrancy**
1		CD 7	Dim
2		CD 7	Dim to Moderate
3		CD 7	Moderate
4		CD 2	Dim
5		CD 56	Dim to Moderate
6		CD 7	Moderate to bright
7		CD 7 / CD 19	Dim, Bright respectively
8		CD 19	Dim
9		CD 7	Moderate to bright

cases with aberrancy have higher mean WBC count (104.35 x 10^9 / L)(13 cases) than those without aberrancy (44.37 x 10^9 / L) (59 cases), cases with aberrancy have higher mean PDW (19.3 μm^3) than those without aberrancy (18.55 μm^3) (T test $P<0.05$, CI=95%) (Figure 1A). Inverse weak correlations between platelets count and PDW, in all acute leukaemic patients ($P<0.05$) (Figure 1B). In B ALL patients, those with negative HLA DR(10 cases) have higher mean PCV (28.75 L/L) than those with positive HLA DR (23.02 L/L) (19 cases) (T test $P<0.05$, CI=95%) (Figure 1C). In AML patients , those with negative CD 117 (10 cases) have higher mean ANC (6.11 x 10^9 / L) than those with positive CD117 (2.37 x 10^9 / L) (25 cases) (T test $P<0.05$, CI=95%)(Figure 1D).

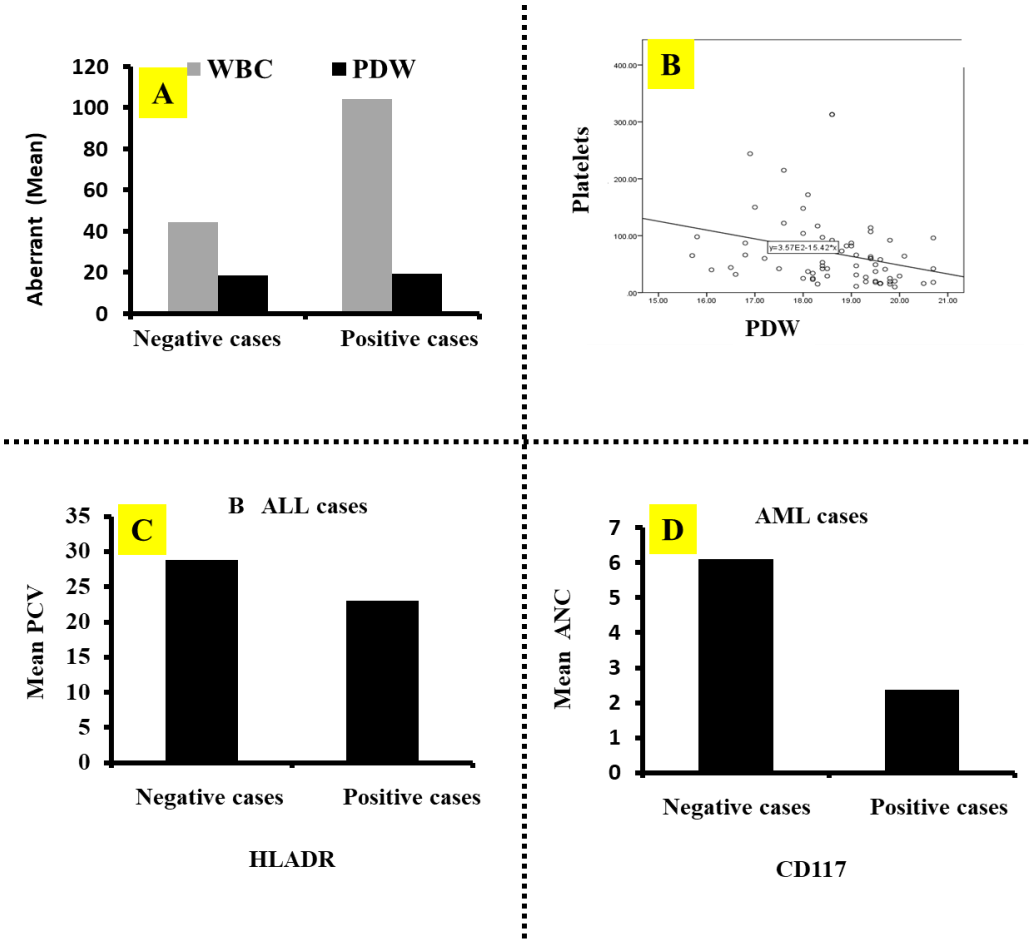

Figure 1. Characterization of CD markers obtained from flow cytometer of positive versus negative cases using WBC, PDW, HLADR, and CD117 and phenotype characterization into either B-ALL or AML cases. (t-test , P< 0.05 , CI= 95%), Platelets count and PDW association are also demonstrated (P< 0.05) .

In ALL patients , those with T ALL(8 cases) have higher mean WBC count (169.68 x 10^9 / L) count and ANC (16.45 x 10^9 / L) than those with B ALL (29 cases) in which mean WBC count (57.13 x 10^9 / L) count and ANC (5.17 x 10^9 / L) , (T test P<0.05, CI=95%) (Figure 2A). In ALL patients , those with positive cCD3(7 cases) have higher mean WBC count (179.84 x 10^9 / L) and mean ANC (17.53 x 10^9 / L) than those with negative cCD3 (30 cases) in which mean WBC count (58.65 x 10^9 / L) and mean ANC (5.29 x 10^9 / L) , (T test P<0.05, CI=95%) (Figure 2B). In ALL patients , those with negative cCD79a (10 cases) have higher mean ANC (14.76 x 10^9 / L) than those with positive cCD79a (27 cases) (4.96 x 10^9 / L) , (T test P<0.05, CI=95%),(Figure 2C). In ALL patients , those with positive TdT (6 cases) have higher mean WBC count (175.17 x 10^9 / L) than those with negative TdT (31 cases) (63.38 x 10^9 / L) ,(T test P<0.05, CI=95%).
(Figure 2D).

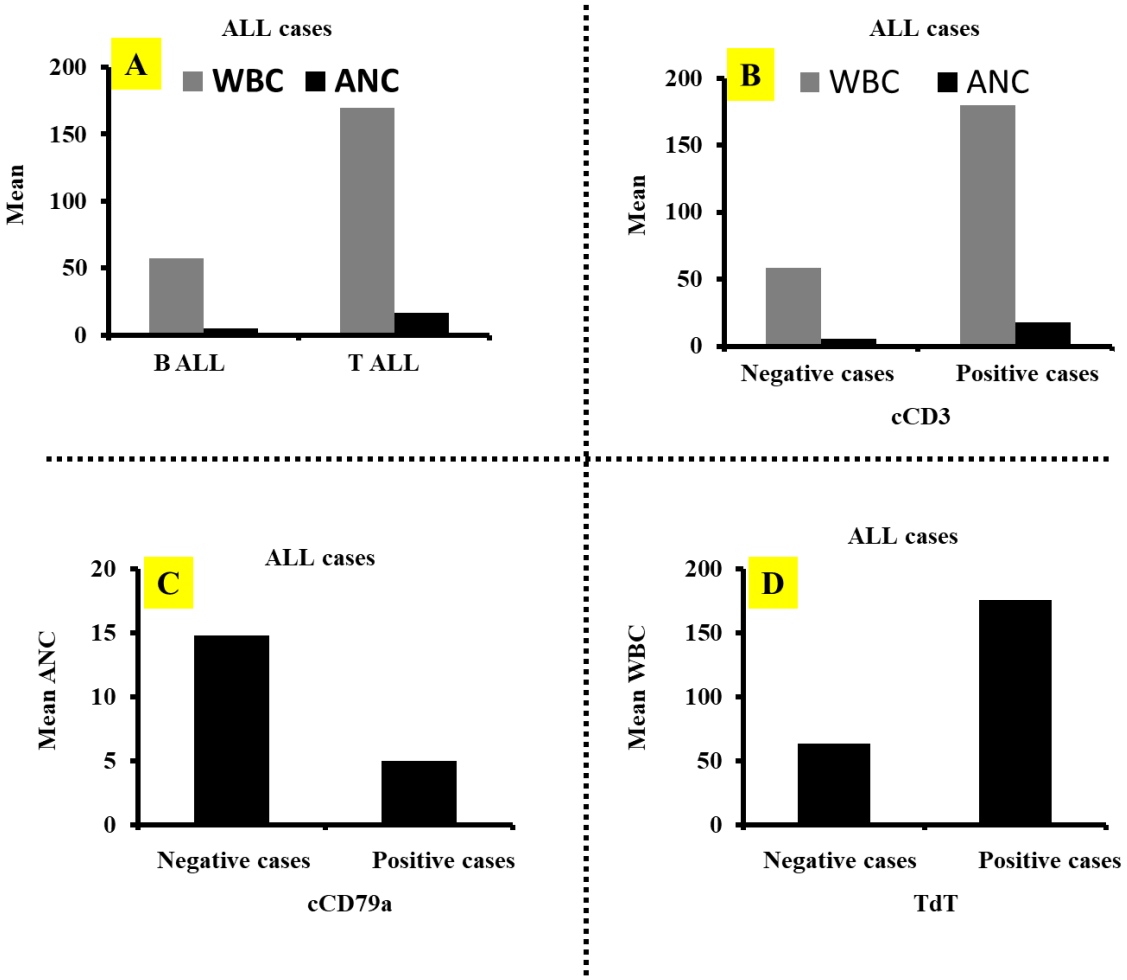

Figure 2. Characterization of CD markers obtained from flow cytometry of positive versus negative cases using WBC, ANC, cCD3, cCD79a, and TdT in ALL cases . (t -test , P< 0.05 , CI= 95%).

DISCUSSION

Numerous studies have provided evidence to support the claim that the mean age is lower in ALL patients compared to AML patients. This observation is of great significance in the field of oncology and has important implications for understanding the epidemiology and clinical characteristics of these two types of leukaemia. Published studies found that the average age of ALL patients was consistently lower than that of AML patients. This finding suggests that age may play a role in the development and progression of different types of leukaemia. By identifying this difference in mean age, researchers can further investigate potential underlying mechanisms that contribute to the divergent age distribution between these two leukaemia subtypes. Additionally, this finding may have implications for diagnostic and treatment strategies, as age-related factors could potentially influence

disease prognosis and response to therapy [1,2] .

In acute leukaemia there is the activation of the coagulation system [7], PDW increased in activation of the coagulation system in acute leukaemia [8] aberrant cases have higher mean PDW so the aberrant cases have more activation of the coagulation system and hence more risk of thrombosis and its complication and worsen disease outcome, in other words, an inferior (poor) prognosis than those cases without aberrancy (Figure 1 A) An assumption could be made that risk of DIC is more in those with aberrancy which may affect the line of management. It is documented in another study that higher PDW may precede activation of the coagulation system[9], another study states that PDW percentage correlates with D dimer level[10], PDW going up with platelets activation going up [8], so platelets activity may be predicted by looking to PDW[11].

In this study, it was observed that the higher mean WBC count in ALL cases was found to be significantly higher in comparison to AML cases. This finding is in agreement with the results of another study in this field which stated that ALL cases have a higher WBC count than AML in a table demonstration [12]. The elevated WBC count in ALL cases may be attributed to the proliferation of lymphoblasts, which are immature white blood cells that rapidly multiply in the bone marrow and infiltrate other organs. Conversely, AML is characterized by the abnormal growth of myeloid cells. These findings are important for diagnosis and treatment strategies, as a higher WBC count may serve as a marker for distinguishing between ALL and AML and has a prognosis importance. Further research are required to confirm these finding and make the underlying mechanisms contributing to the differences in WBC counts between the two types of leukaemia more clear [8,11].

Positive HLA DR is considered a sign of immaturity since HLA DR is expressed by uncommitted haemopoietic stem cells[5] This study suggests that these cells are immature cells, the more immature bone marrow the more bone marrow suppression the less ability to produce mature blood elements (red blood cells, white blood cells and platelets) this may explain the lower mean PCV in positive HLA DR cases of B-ALL (Figure 1 C), our suggestion may be supported by a study demonstrating an association of HLA DR expression and proliferating blast in ALL , meaning ALL with blast of HLA DR expression have more proliferative capacity and these abnormal cells will suppress the normal hemopoietic bone cells resulting in less production of red and white blood cells and less platelets [13].

CD 117 is considered a sign of immaturity [5], in this study, AML cases with positive CD 117 have lower mean ANC, we suggest that AML cases with positive CD117 have more immature bone marrow compartments (blasts) than those with negative CD 117, so the mature blood cell (Red blood cells, WBC and platelets) is expected to be less, mean ANC in this study is a statistically approved demonstration (Figure 1 D). In a study, CD 117 is promoting AML cells (malignant cells) proliferation leading to increasing immature (malignant) cells compartment and increasing suppression of normal hemopoietic cells leading to decreasing production of red and white blood cells and less platelets, CD 117

expression is a well known poor prognostic marker in AML [14], these facts support our results.

In (Figure 2 A), it is observed that the means of ANC and WBC counts are higher in T-ALL compared to B-ALL. This difference in ANC and WBC counts suggests that there is greater preservation of bone marrow function, and so more ability to proliferate in T-ALL as compared to B-ALL. This could potentially be due to differences in the underlying biology and cellular characteristics of T-ALL and B-ALL [15]. Further research is required to elucidate the specific mechanisms responsible for this observation and to understand the clinical implications of these findings. After an extensive search a comparable studies could not be found to verify our results, nonetheless, this finding highlights the importance of considering the variations in bone marrow function and immune cell production in different types of leukaemia, as it may have implications for disease progression and treatment strategies .

In ALL patients (Figure 2B), those with positive cCD3 have higher mean WBC count and mean ANC than those with negative cCD3, the explanation is that cCD3 is a specific T-ALL marker, T-ALL have more efficient bone marrow compared with B-ALL as stated above. Since cCD79a is a specific B-ALL and cCD79a is an indicator of immaturity [5], it is expected to notice a higher mean ANC in those with negative cCD79a (Figure 2 C).

Suppress TdT expression (as in negative cases for TdT) leads to decreased growth, and apoptosis, of T and B cells this supports the result in (Figure 2 D) which states a negative TdT group among ALL patients has a lower mean WBC count[16], our results are in agreement with other study in which B-ALL with positive TdT have more frequencies of high WBC count [17]. This indicates that inhibiting TdT expression may be a therapeutic option for ALL patients.

In this study (Table 1) CD 7 is the most frequent aberrant phenotype expressed in AML cases, others say it is CD19 [18]. some authors consider AML with CD7 expression as a separate clinical group with more chance to have CNS disease and hepatomegaly with more incidence in the young age group [19], CD7 is a bad prognostic indicator [20,22], CD7 expression in AML cases associated with noticeable higher WBC count than the patients without this expression [23], CD56 expression in AML also have a negative prognostic impact [22]. Another study performed in Pakistan supported our result that CD 7 is the most frequent aberrant CD marker expressed in AML [24].

While it may be challenging to gather many newly diagnosed leukaemia patients due to limited resources, efforts should be made to collaborate with multiple medical institutions and research centers to increase the sample size and enhance the statistical power of the studies. By doing so, researchers can obtain more useful and meaningful results that can potentially have a significant impact on the field of leukaemia research and patient care.

CONCLUSION

Acute leukemia with aberrant phenotypes cases is suspected to be more prone to have coagulopathy, an inferior prognosis. Leukaemic patients with the absence of some markers of immaturity (cCD79a, CD117) have higher ANC which may affect disease management. In ALL cases inhibiting TdT expression may be a therapeutic option.

CONFLICT OF INTEREST

No Conflict of interest regarding this article.

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