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Aberrant Expression of CD2 in cases with *de novo* Acute Myeloid Leukemia Blast Cells

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Abstract

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Background. Acute myeloid leukaemia (AML) is a malignant clonal disorder of immature cells in the haemopoietic hierarchical system. Flow cytometry (FC) immunophenotyping is essential for diagnosis and subtyping of AML. CD2 antigen is expressed on thymocytes, peripheral T cells, NKCs, and a subpopulation of B cells; it is aberrantly expressed in a quarter of cases of acute promyelocytic leukaemia.

Aims. This study is designed to investigate the frequency of the aberrant CD2 expression on adult AML blast cells at the initial diagnosis, and correlate their expression with complete blood count (CBC) results, blast cells percent in peripheral blood (PB) and bone marrow (BM), the AML subtypes, extramedullary manifestations, and the initial response to the induction therapy.

Methods. Thirty adult cases (> 15 years) with *de novo* AML were consequently selected in the period from the 5th of October 2023 to the 10th of March 2024. Their mean age was 38.97±18.59 yrs (18 males and 12 females). All AML cases were diagnosed according to the WHO criteria for AML and they were diagnosed by cytomorphology and FC for suspected cases. Aberrant antigens CD2 expressions was investigated by eight-color (BD FACSCanto II USA) at diagnosis.

Results. CD2 was expressed on blast cells in 13.3% of AML cases. AML cases with positive CD2 expression were younger than those with negative expression and CD2 was expressed equally by males and females. CD2 was significantly more expressed on leukaemic cells of M3 cases. There was no significant correlation with any of the haematological parameters with CD2 expression. Also it appeared that there were no significant correlations between CD2 aberrant expression with the extramedullary manifestations and with the non-response to the induction therapy.

Conclusion. Aberrant CD2 is significantly more expressed on leukaemic cells of M3 cases. Neither CD2 aberrant expression is found to be significantly correlated with the age, gender, nor with the haematological parameters, induction therapy response or extramedullary manifestations.

Introduction

Leukaemic transformation is assumed to occur in many cases at, or near, the level of the haemopoietic stem cell before it has been embarked on any lineage commitment. Some cases may originate at a slightly later stage in cells that are committed to lineage differentiation. The proliferative advantage of the leukaemic stem cell coupled with impairments in differentiation and inhibition of apoptosis lead to accumulation of immature or blast cells in the BM. The blasts eventually suppress normal hematopoiesis leading to marrow failure and infiltrate other organs and tissues [1, 2].

Accurate diagnosis and classification in AML are essential for treatment decisions and assessment of prognosis. Initial assessment requires a careful history, physical exam, CBC with PB smear review, BM examination, FC, cytogenetics, and selected molecular genetic analyses [3].

Examples of aberrant immunophenotype is CD2 which is expressed in a quarter of cases of M3 AML and is occasionally expressed in other subtypes and [4]. CD2 antigen is expressed on thymocytes, peripheral T cells, and NKCs, it is also expressed on a subpopulation of B cells. It is aberrantly expressed in a quarter of cases of M3 AML and is occasionally expressed in other subtypes. Evidence indicates that AML cases with blasts that express surface CD2 have a poor response to therapy and might benefit from alternative therapy [4,5].

The peak incidence of AML rate occurs in the first year of life and then decreases steadily up to the age of 4 years and remain relatively constant in childhood and early adulthood. AML is thus a disease of older adults and it is rarely diagnosed before the age of 40 years. Cases newly diagnosed with AML have a median age of 65 years; thereafter, the incidence increases progressively with age. AML represents approximately 90% of all acute leukaemias in adults but accounts for only 13% of leukaemia cases in children younger than 10 years. The incidence of AML varies with gender and race, it is higher in males than in females and in whites than in blacks. Incidence rates are greater in developed countries and in industrialized cities. Studies reveal an increased risk for Eastern European Jews and a decreased risk for Asian populations [6].

In Iraq-Baghdad; during the year 2006, AML in adults was 27.5% of acute leukaemias and this result was agreed with the observations in the North of Iraq. Males with AML were 61.1% and females were 38.9%. The peak incidence was at age 25-34 years [7].

CD2 is a glycoprotein of approximately 50 kDa, a member of the immunoglobulin superfamily; expressed on thymocytes, all T lymphocytes, large granular lymphocytes, and NKCs. Evidence indicates that AML cases with blasts expressing surface CD2 antigen have a poor response to therapy [4, 8]. CD2 is aberrantly highly expressed in AML-M4Eo, this expression stimulates proliferation of the leukaemic cells, which might explain the high WBC count often found in this type of AML. In APL with cytogenetic evidence of t(15;17)(q22;q21), CD2 expressed more often in M3v than in M3. CD2+ve APL associated with leukocytosis, shorter CR duration, and a trend toward shorter OS than CD2-ve APL [9].

Fit cases (< 60 years, selective cases up to age 75 year) receive intensive therapy. Treatment includes induction therapy and post-remission therapy (consolidation). Non-low risk-cases are evaluated for stem cell transplantation (SCT) in first remission. Less fit cases ≥ 60 years, or younger cases with significant comorbidities) receive low-intensity therapy [10].

This study is designed to investigate the aberrant CD2 expression frequency on adult AML blast cells at initial diagnosis, to correlate the expression with the CBC results, blast cells percent in PB and BM and with the WHO classification subtypes, extramedullary manifestations, and the initial response to the induction therapy.

Methods

Design and setting

This prospective cohort study was conducted on thirty adults newly diagnosed AML cases from the 5th of October 2023 to the 10th of March 2024. Their mean age was 38.97 ± 18.59 (mean ± SD), 18 of them were males and 12 were females.

The cases were admitted to the Haematology Department of Baghdad Teaching Hospital of the Medical City. The Cases PB, bone marrow aspirate (BMA) samples were analyzed in the Teaching Laboratories of the Medical City in Baghdad. FC was done by eight-color (BD FACSCanto II, USA) in the Teaching Laboratories of the Medical City in Baghdad.

For each case a data was collected including: name, age, sex, main symptoms and physical signs especially the presence of extramedullary manifestations which include lymphadenopathy, splenomegaly, hepatomegaly, gingival hypertrophy, skin infiltrates and involvement of CNS [11], and evaluate patients' response to the induction therapy.

Inclusion Criteria

1. All AML cases were above 15 years old.

2. All AML cases were newly diagnosed.

Therapy and Follow-up

All cases were evaluated for CR achievement three weeks after one cycle of chemotherapy according to Cheson et al. definition [12]. For remission induction, the cases were categorized into high and low risk groups by according to their age and WBC count at presentation (risk stratification); so those cases with age ≥ 60 years and WBC count ≥ 50×10^9/L at presentation were considered as a high risk and given less intensive therapy; while those cases with age < 60 years and WBC count < 50×10^9/L at presentation were considered a low risk and received the induction therapy protocol [13,14].

Blood sampling

A total venous blood sample of 2.5 ml and BM aspirate sample of 0.5 ml were obtained from each case included in this study by venipuncture from antecubital fossa or BMA from posterior superior iliac crest under aseptic technique, and the samples were collected in K2-EDTA tubes. All were examined for CBC by automated device (XN-1000, Sysmex, Japan). The PB and BM smears were examined by a specialist haematologist for blasts percentage and assessing the remission after induction therapy. Blood films were done; they were stained by Leishman's. BM smears were done by making films, 3–5 cm in length, of the aspirated marrow using a smooth-edged glass spreader. The marrow fragments are dragged behind the spreader and a trail of cells left behind them. After thorough drying, the films of BM were stained as for PB films.

Flow cytometric Immunophenotyping

After AML cases have been confirmed, the samples were transferred to the flow cytometry unit to be investigated for the aberrant expression of surface marker antigen CD2 (6 hours being the maximum time since obtaining the sample) by using eight-color flow cytometry device (BD FACSCanto II, USA). Gating of the cells of interest was depending on SSC/CD45 gate. The device software is based on the FACSDiva™ Clinical Software operating system for multi-parametric data acquisition, display, data analysis, and instrument control. Flow cytometry (BD FACSCanto II, USA) is used as a fully-equipped FC with laser excitation in blue, red, and violet, it analyses up to three optical parameters (FSC, SSC, and 8 fluorescence channels) [15].

Determination of the Aberrant Phenotype

Identification of blast cells was performed using SSC versus CD45 parameters. Basically, antigen expression is considered to be positive when the percentage of positive blast cells is equal or greater than 20%. Similarly, aberrant phenotypes are defined when at least 20% of the blast cells expressed that particular phenotype [18].

Statistical Analysis

SPSS (Statistical Package for Social Science; version 16) was used to analyzed findings. Numeric variables were presented as mean, median, SD, SE, and range. Nominal variables were expressed as number and percentage. Pearson's chi-square and Fisher exact tests were used to evaluate difference between groups. Independent sample student t-test was used to compare the mean of numeric variables between groups. The level of ($P<0.05$) was considered significant.

Results

Patients age and sex

The mean age of AML cases included in this study was 38.97±18.59 (median=37.5 years). AML cases were observed more in males (60%) than in females (40%).

Correlation between the aberrant CD2 expression with age

Regarding CD2, those cases with a positive expression were slightly younger (mean age 37±12.83 years) than those with a negative expression (mean age 39.27±19.51 years). Also there was no significant correlation between this expression and mean age of the case (Table 1).

Table 1. Aberrant CD2 expression with the age

	CD2 negative			CD2 positive			P-value
Parameter	Mean	SD	SE	Mean	SD	SE	
Age	39.27	19.51	3.83	37.00	12.83	6.42	0.825

Correlation between the aberrant CD2 expression with gender

Tables (2) showed that there was no significant correlation between the gender and the aberrant CD2 expression.

Table 2. Aberrant CD2 expression with gender

	Negative CD2		Positive CD2		Total	
Gender	No.	%	No.	%	No.	%
Female	10	83.33	2	16.67	12	100.00
Male	16	88.89	2	11.11	18	100.00
p-value = 1.000						

Correlation between the aberrant CD2 expression with the haematological parameters.

Table (3) showed that the total WBC count, PB and BM blast cells percentage of AML cases with aberrant CD2 expression were non-significant.

Table 3. Aberrant CD2 expression with haematological parameters

Parameter	CD2 negative			CD2 positive			P-value
	Mean	SD	SE	Mean	SD	SE	
Total WBC ($\times 10^9$/L)	63.86	75.98	14.90	106.20	112.35	56.17	0.337
Hb (g/dl)	7.56	2.05	0.40	7.30	0.20	0.10	0.803
Platelet count ($\times 10^9$/L)	82.27	106.05	20.80	40.50	33.00	16.50	0.447
PB blast cells %	63.27	28.29	5.55	72.00	19.82	9.91	0.559
BM blast cells %	72.38	24.24	4.75	55.25	23.84	11.92	0.198

The distribution of aberrant CD2 expression in relation to AML FAB classification

CD2 was expressed more on M3 subtype (2/3, 66.7%) with statistically significant correlation (p-value = 0.039). It is also expressed in M2 and M5 (1/9 and 1/8, respectively). CD2 is not expressed on M1, M4 and M6 subtypes (Table 4).

Table 4. Aberrant CD2 expression with the AML FAB subtypes

FAB subtype	CD2			
	Positive	Negative	Total	P-value
M1	0	7	7	0.548
M2	1	8	9	1.000
M3*	2	1	3	**0.039***
M4	0	2	2	1.000
M5	1	7	8	1.000
M6	0	1	1	1.000

Correlation between the aberrant CD2 expression with the extramedullary manifestations

Table (5) showed that there is no significant correlation between CD2 aberrant expression and the extramedullary manifestations.

Table 5. Aberrant CD2 expression with the extramedullary manifestations

Extramedullary manifestations	CD2				Total	
	Negative		Positive			
	No.	%	No.	%	No.	%
Yes	7	26.9	1	25	8	26.67
No	19	73.1	3	75	22	73.33
Total	26	100	4	100	30	100.00

Correlation between the aberrant CD2 expression with CR achievement

For cases with aberrant CD2 expression 1 out of 4 did not respond to induction treatment, and it appeared that there was no significant correlation between this expression with the non-responsiveness to induction therapy (Table 6).

Table 6. Aberrant CD2 expression with CR achievement

CR achievement	CD2					
	Negative		Positive		Total	
	No.	%	No.	%	No.	%
Yes	16	61.5	3	75	19	63.3
No	10	38.5	1	25	11	36.7
Total	26	100	4	100	30	100.00

Discussion

In this study, the CD2 was expressed in 13.3% of AML cases. This result is comparable to that obtained by Lewis RE et al. [19] who reported CD2 expression in 16.7% of AML cases, Zheng J et al. [20] in 12.5%, Bahia DM et al. [18] in 11.4%, and Auewarakul CU et al [21] in 8.7%. In contrast, this study result was higher than that reported by Jiang et al. [22] 4.9%, and lower than that of Lin et al. [23] 23%.

The mean age of CD2+ cases was lower (37.0±12.83 years) than that of cases with no CD2 expression (39.27±19.51 years). Also no significant age difference was identified between the two groups (p= 0.825). This result is comparable to that obtained by Zheng J et al. [20] and Wang XB et al. [24] but differs from that obtained by Ball ED et al. [25] who reported that CD2+ cases were older than those CD2− cases.

CD2 expressed equally in relation to gender with an M:F ratio of 1:1 without any significance, this result is comparable to that of Ball ED et al. [25] and Xu F et al. [26], who also found no significant correlation between CD2 expression and gender of AML case.

The hematological parameters that had been studied included Hb, total WBC count, platelet count, PB and BM blast cells percent. There was no significant correlation between the aberrant CD2 expression and any of the haematological parameters studied. This result is comparable to that of Ball ED et al. [25], Smith FO et al. [27] However, Xu F et al. 2014 [26] proposed that CD2 expression on APL leukaemic cells correlated significantly with a higher pretreatment total WBC count.

CD2 was expressed more on M3 subtype (2/3, 66.7%) and this expression reached the level of significance (p= 0.039) in agreement with Zheng J et al. [20], Auewarakul CU et al. [21] and Wang XB et al. [24]. However, Khalidi HS, [28] showed that this expression did not reach the level of significance.

For CD2 aberrant expression, there was no significant correlation with the extramedullary involvement in agreement with Ball ED et al. [25] and Chang H et al. [29] while Ortolani C, [30] published that the presence of CD2 on AML blasts is associated with an increased risk of extramedullary disease.

In this study, there was no significant correlation between CD2 expression with CR achievement. In contrast, most of the studies worldwide found a correlation, e.g. Bradstock K et al. [31] and Ferrara F et al. [32] found that CD2 expression in AML was significantly

associated with lower CR rate, while Ball ED et al. [25] described a high CR rate in adult AML cases expressing CD2. The reason for this discrepancy may be due to different study population and the low number of cases in our study.

Conclusion

Aberrant CD2 is significantly more expressed on leukaemic cells of M3 cases. Neither CD2 aberrant expression is found to be significantly correlated with the age, gender, nor with the haematological parameters, induction therapy response or extramedullary manifestations.

Authors' contributions

Conceptualization; Data Curation; Investigation; Methodology; Project administration; Resources; Software; Writing – original draft and Writing – review & editing

Funding

None.

Conflicts of interest

The authors declare no conflict of interest regarding this article.

Ethical approval

The Medical Ethical Committee of the Medical City of Baghdad, approved this study. Participant consent was waived by the committee since only patient files were reviewed.

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