Aberrant Expression of CD56 in Hematologic Malignancies

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ABSTRACT

Introduction: Hematologic malignancies (HMs) are a wide range of diseases with varying rates, prognoses, and origins resulting from bone marrow or lymphatic system cells transforming into malignant cells. The typical phenotypic marker of natural killer cells, CD56, is expressed by various immune cells, including monocytes, dendritic cells, alpha and beta T cells, and gamma and delta T cells. These CD56-expressing cell types have potent immunostimulatory effector capabilities, such as generating T helper 1 cytokines and effective cytotoxicity. So, we aimed to look for ectopic expression of CD56 in different hematologic malignancies and to find out if there is a possible diagnostic or prognostic value for CD56.

Patients and Methods: The study involved 55 patients with hematologic malignancies. The patients were diagnosed after complete blood count, bone marrow aspirate (BMA), bone marrow biopsy (BMB), and Immunophenotyping.

Results: AML cases showed ectopic expression of CD56 (25.7%), T-ALL and MM cases showed (100%), cases of B-ALL showed (12.5%), and cases of CLL and Lymphoma were without ectopic expression of CD56.

Conclusion: CD56 does not show an impact on the severity of disease but may be related to response to treatment, relapse rate, prolonged survival of tumor cells, and apoptosis of cells or survival of patients, which need further prospective studies.

Keywords: Hematologic malignancies; cluster of differentiation 56; bone marrow aspirate.

Introduction:

Hematologic malignancies (HMs) are a wide range of diseases with varying rates, prognoses, and origins resulting from bone marrow or lymphatic system cells transforming into malignant cells[(1)].

The typical phenotypic marker of natural killer cells, CD56, is expressed by various immune cells, including monocytes, dendritic cells, alpha and beta T cells, and gamma and delta T cells. These CD56-expressing cell types have potent immunostimulatory effector capabilities, such as generating T helper 1 cytokines and effective cytotoxicity. Surprisingly, individuals with various infectious, autoimmune, or neoplastic diseases have been found to have phenotypic changes and functional deficits of the CD56 immune cell fraction. The term neural cell adhesion molecule (NCAM) is another name for CD56 [(2)].

This research aimed to look for ectopic expression of CD56 in different hematologic malignancies and to find out if there is a possible diagnostic or prognostic value of CD56.

Patients and Methods:

Patients:

The time frame for conducting this study was from September 2020 to December 2022 at Assuit University's Clinical Pathology Department. The study involved 55 patients who came to Assuit University Hospital with hematologic malignancies (AML, ALL, CLL, lymphoma, multiple myeloma, and bone marrow secondaries). Bone marrow aspirate (BMA), bone marrow biopsy (BMB), and Immunophenotyping were used to diagnose the patients.

Methods:

1. Full clinical evaluation including:
   - Full history taking including (age, sex, therapeutic history, transfusion).
   - Clinical examination including: anemic manifestation, fever, hepatomegaly, splenomegaly, lymphadenopathy, bleeding tendency, and bone tenderness.

2. Laboratory investigations including:

Complete blood count: Immediately after drawing two milliliters of venous blood into EDTA, peripheral blood smears were spread out for Leishman staining and differential leucocytic counting. The ADVIA 2120i (Siemens, Germany) was used for CBC.

BMA: Samples were taken from the anterior or posterior superior iliac spine in EDTA tubes for each patient. Immediately after, half a milliliter of BM aspirate was combined and placed on glass slides. Smears were then spread out to be inspected after Leishman staining. Using a gentle dispense, one milliliter of BM aspirate was placed into an EDTA solution tube for immunophenotypic analysis using flow cytometry (FCM). The FCM sample was analyzed in less than a day and kept at room temperature. Leishman-stained BM aspiration smears were examined for cytochemical analysis using Sudan black B, Nonspecific esterase, and Periodic acid Schiff, as well as morphological BM proliferation and BM cellularity assessment.

Immunophenotyping utilized monoclonal antibodies (MoAbs) from BD and Immunostep on a Becton-Dickinson fluorescence-activated cell sorter (BD FACSCalibur).

Procedure:

The lab number and staining monoclonal antibody were written on plastic test tubes for each patient. Next, well-mixed EDTA anticoagulated whole blood and bone marrow aspirate were added to 10µL of monoclonal antibodies. The tubes were then gently mixed, capped, and incubated for 15 minutes at room temperature in the dark. Finally, a lysing solution was added to the tubes, which were inverted once.

Next, the tubes were sealed, shaken gently to mix the contents, and then incubated for 15 minutes at room temperature in the dark. After that, the tubes were centrifuged for 3 minutes at 2500 rpm, and the supernatants were removed. Next, phosphate buffer solution was added to the tubes, which were centrifuged for 3 minutes at 2500 rpm. Finally, the supernatants were removed and transferred back into sheath fluid for instantaneous analysis using multicolor flow cytometry (FACS Calibur). Prepared samples were analyzed within 2 hours or refrigerated in the dark for analysis within 24 hours if circumstances were unsuitable. Expression of CD56 was studied with another marker to show that it is ectopically expressed and belongs to NK cells.

Ethical Considerations:

The study was approved and monitored by the Medical Ethics Committee of the Faculty of Medicine-Assiut University, IRB No. 17101335.

Statistical Analysis:

SPSS (statistical package for the social sciences; SPSS Inc., Chicago, IL, USA) version 22 was used for all statistical computations. When not regularly

distributed, quantitative data were statistically reported using mean ± SD or median (range). When applicable, relative frequencies (percentages) and frequencies (number of cases) were used to characterize qualitative data statistically. Since the data were not normally distributed, the Mann-Whitney U test and the Kruskal Wallis test were used to compare the quantitative variables. The Spearman rho correlation test was used to determine the correlation between different variables. At the 0.05 level, the P-value is always two-tailed and significant.

Results:

The study included 55 patients with different hematologic malignancies. Their age ranged from 4 to 77 years old, with SD (42.65 ± 18.69) years. More than half (67.3%) of the studied cases were males, and 18 cases (32.7%) were female, with a male-to-female ratio of 2.1:1.

As regards the age and sex of the studied cases, no significant difference was observed in comparing the level of CD56 expression according to age and sex (P=0.290 and 0.993), respectively.

Regarding the clinical presentation among the studied cases, they were presented with anemic manifestations, fever, bleeding tendency, bone pain, generalized fatigue, cough, weight loss, headache, and abdominal distension on clinical examination; some of them had splenomegaly, hepatomegaly, and lymphadenopathy.

The most frequent diagnosis in the current study was acute myeloid leukemia (63.6%), B-cell acute lymphoblastic leukemia (14.5%), T-cell acute lymphoblastic leukemia (3.6%), Lymphoma (5.5%), chronic lymphoblastic leukemia (7.3%), multiple myeloma (3.6%) and BM secondaries (1.8%) respectively, (**Table 1**).

Table (1): Diagnosis of the studied cases

Diagnosis	N	(%)
AML	35	(63.6%)
B-ALL	8	(14.5%)
T-ALL	2	(3.6%)
CLL	4	(7.3%)
Lymphoma	3	(5.5%)
MM	2	(3.6%)
BM secondaries	1	(1.8%)

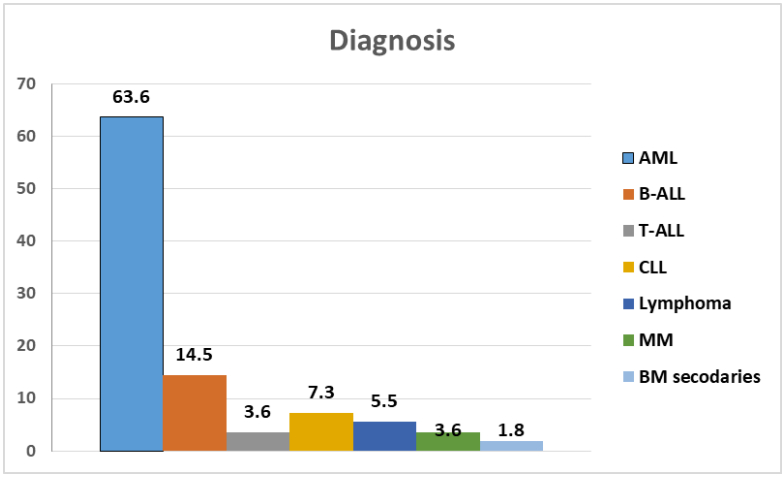

Fig. (1): Bar graph showing the diagnosis of the studied cases. Mean CD 56 expression was 15.13 ± 23.85 with a range between 25.5% and 74.5%.

Table (2): CD56 expression pattern among the studied cases

CD56	N=55	
Median (range)	3.18 (0.10 - 88.40)	
Mean ± SD	15.13 ± 23.85	
Negative (<15.0%)	41	(74.5%)
Positive (≥15.0%)	14	(25.5%)

The maximum percentage of normal NK cells is 15%. If it is more than 15%, there is an NK tumor or ectopic expression of CD56. Roughly 15% of all circulating lymphocytes are NK cells [(3)].

Relations between CD56 expression and the diagnosis of studied cases revealed that AML cases showed a median of 2.64 and ranged from (0.10 - 88.40) and P-value < 0.05, T-ALL cases showed a median of 79.16 and ranged from (77.73 - 80.60), B-ALL cases showed a median of 3.06 and ranged from (0.86 - 28.01), CLL cases showed a median of 2.93 and ranged from (1.43 - 10.80), lymphoma cases showed a median of4.37 and ranged from (0.74 - 7.99), multiple myeloma cases showed a median of 56.96 and ranged from (42.92 - 71.00) and in case of BM secondaries showed a median of 0.29.

Correlations between CD56 expression and hematological laboratory data of the studied cases were insignificant except in monocytes.

Table (3) shows that 35 AML cases (74.3%) were CD56 negative, 9 cases (25.7%) were CD56 positive (P=0.033), eight B-ALL cases (87.5%) were CD56 negative, and one case (12.5%) was CD56 positive, the two cases with T-ALL had positive CD56 expression, four cases with CLL had negative CD56 expression, the three cases with Lymphoma had negative CD56 expression, the two MM cases had positive CD56 expression and one case with BM secondaries had negative CD56 expression.

Table (3): Relations between CD56 expression and the diagnosis of studied cases

	Negative (n=41)		Positive (n=14)		P-value
Diagnosis					0.049
- AML	26	(74.3%)	9	(25.7%)	
- B-ALL	7	(87.5%)	1	(12.5%)	
- T-ALL	0	(0.0%)	2	(100.0%)	
- CLL	4	(100.0%)	0	(0.0%)	
- Lymphoma	3	(100.0%)	0	(0.0%)	
- MM	0	(0.0%)	2	(100.0%)	
- BM secondaries	1	(100.0%)	0	(0.0%)	

Significance defined by $p < 0.05$

No significant relation was observed between CD56 expression and laboratory data of the studied AML cases (P>0.05, for all), except that the BM monocytes were significantly higher among AML cases with positive CD56 expression (median was 0.0 (range; 0.0 - 5.0) among negative CD56 expression vs. 1.0 (range; 0.0 - 15.0) vs. among positive CD56 expression, P=0.015).

Discussion:

Hematologic malignancies (HM) are a broad category of illnesses with varying reasons, occurrences, and prognoses [(4)].

As an isoform of the neural cell adhesion molecule (NCAM), the cluster of differentiation 56 is a member of the immunoglobulin supergene family [(5)]. This antigen facilitates cell communication and may have a role in cell-mediated cytotoxicity [(6)].

This study aimed to detect ectopic expression of CD56 in different hematologic malignancies. The study included cases with the following diagnoses: AML, T-ALL, B-ALL, CLL, MM, and Lymphoma. Correlations between cases with ectopic expression of CD56 and (age and sex) were not significant.

Correlations between cases with ectopic expression of CD56 and (anemia, blast cell count, and thrombocytopenia) did not show an impact on the severity of the disease.

AML cases show ectopic expression of CD56 (25.7%); in other research, the percentage ranged from (15.5% to 21.7%) [(7-10)]. Correlations between AML cases with ectopic expression of CD56 and (anemia, blast cell count, and thrombocytopenia) did not show an impact on the severity of the disease. Still, it may be related to response to treatment, relapse rate, prolonged survival of tumor cells, apoptosis of cells, or survival of patients, which need further prospective studies.

It is generally acknowledged that the expression of CD56 is an aberrant marker to identify abnormal monocytes in these disorders since a high frequency of CD56 expression has been documented on monocytic cells in acute and chronic monocytic leukemia [(11)].

This ectopic expression of CD56 in myeloid cells might be due to genetic rearrangement (t(9;11)(p21.3;q23.3); MLLT3-KMT2A) according to WHO classification 2016 [(12)], as The NCAM gene is found on chromosome 11q23; chromosomal breaks have frequently been seen at this site in a variety of leukemias [(13)], which need further studies.

T-ALL cases show 100% ectopic expression of CD56, while in other research,

the percentage ranged from (13.9% to 18.9%) [14 -16].

Ectopic expression of CD56 in T-ALL might be due to chromosomal rearrangements in KMT2A (MLL) on chromosome 11q23, resulting in ectopic expression of transcription factors or the generation of fusion genes with transcriptional/epigenetic activity [17], 11q23/MLL. Approximately 4% of adolescents with T-ALL and 6% to 8% of adults with the illness had abnormalities of 11q23. T-ALL was found in 40 out of 459 (8.7%) individuals in an international collaborative investigation of ALL with an 11q23 abnormality and a recognized immunophenotype. Though cases with a del(11)(q23) should be carefully investigated by FISH utilizing the MLL probe to rule out a subtle translocation such as t(6;11)(q27;q23), most patients with T-ALL and 11q23 rearrangements have a t(11;19)(q23;p13.3). Infrequently observed translocations in T-ALL include t(9;11)(p22;q23), t(10;11)(p12;q23), and (4;11)(q21;q23), in addition to those observed in B-lineage ALL or AML [18].

Cases of MM show 100% ectopic expression of CD56, while in other research, the percentage of ectopic expression of CD56 ranged from (62% to 85%) [19-22]. This difference may be related to the small number of cases. No paper is available about chromosomal rearrangements on chromosome 11q23 in multiple myeloma.

Cases of CLL were without ectopic expression of CD56, which may be due to deletions at 11q23 in CLL that were detected by Molecular studies using fluorescence in situ hybridization (FISH) with the YAC probe 755b11. These deletions are the second most common genetic abnormality in ~20% of CLL cases [23].

Lymphoma cases did not show ectopic expression of CD56; in other research, the percentage of ectopic expression of CD56 was 18% [24], as chromosomal abnormalities involving 11q23 were found in 49% of the mantle cell lymphoma cases in 21% of chronic lymphocytic leukemia/small lymphocytic lymphoma cases, and 24% of diffuse large- cell lymphoma. However, these 11q23 abnormalities that occur spontaneously in NHL are usually deletions or point mutations and do not involve the MLL gene [25].

Cases of B-ALL showed ectopic expression of CD56 (12.5%); in other research, the percentage of ectopic expression of CD56 ranged from (12% to 5.1%) [26-28], which may be related to genetic rearrangement in t(9;11)(p22;q23) mainly occurs in acute non-lymphoid leukemia (ANLL) but occur in early B-ALL[29].

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