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Study of CD25 expression on leukemic cells: a prognostic factor in acute myeloid leukemia

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Abstract

Background: Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy characterized by a clonal expansion of myeloid blasts. Treatment strategies of patients with AML are based on various prognostic factors, including age and performance status of the patient, as well as cytogenetic and molecular characteristics of the leukemic clone.

Our aim was to study the expression of cluster of differentiation (CD)25 in adult Egyptian patients with newly diagnosed AML and to assess its prognostic relevance.

Methods: This study was conducted on 50 newly diagnosed AML patients at the Hematology Unit, Internal Medicine Department, Alexandria Main University Hospital. All patients were subjected to full history taking, thorough clinical examination, and laboratory investigations, including detection of CD25 expression on blast cells by flow cytometry. Conventional karyotyping was done on 11 patients at the time of diagnosis.

Results: In our study group, 12 patients were positive for CD25 expression, and this positivity was associated with worse overall survival and shorter leukemia-free survival. On evaluating the response to treatment among CD25-positive AML patients with normal karyotype, they had lower complete remission rates and higher relapse and death rates.

Conclusions: Expression of CD25 in AML patients at presentation can be considered a poor independent prognostic factor.

Keywords:
acute myeloid leukemia, prognosis, CD25 expression, cytogenetics, flow cytometry

Introduction

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy characterized by the clonal expansion of myeloid blasts in the peripheral blood, bone marrow, and/or other tissues, with maturation arrest of cells in the bone marrow and impaired production of normal blood cells [1, 2].

Treatment strategies of patients with AML are based on various prognostic factors, including age and performance status of the patient, as well as cytogenetic and molecular characteristics of the leukemic clone [3].

To date, cytogenetic abnormalities have provided the most prognostic information [4]. However, 40%-49% of adult AML patients have a cytogenetically normal (CN) phenotype, which would make additional prognostic factors particularly useful in this group [5, 6].

Cluster of differentiation (CD)-25 represents the a-chain of the interleukin-2 receptor (IL-2Ra), a low-affinity binding receptor [7]. The IL-2 receptor is composed of different combinations of three subunits (alpha, beta, and gamma chains) and is normally expressed on activated T-cells. Upon binding its ligand IL-2, the IL-2 receptor induces T-cell proliferation and differentiation [8].

Recent research has described CD25 as a poor prognostic factor in acute lymphoblastic leukemia [9]. Moreover, in AML, previously published data from small retrospective studies have suggested an unfavorable impact of CD25 [10, 11].

Therefore, our aim was to evaluate the expression of CD25 in adult Egyptian patients with newly diagnosed AML and thereafter study its impact on prognosis.

Subjects and methods

The current study was conducted with 50 newly diagnosed adult AML patients before receiving induction chemotherapy. All patients were presented to the Hematology Department of the Alexandria Main University Hospital. Complete blood count (CBC) was determined and bone marrow (BM) aspirate was obtained on initial diagnosis and 28 days after receiving induction chemotherapy to follow up their response to treatment (complete remission [CR] or relapse), and we evaluated the survival for 18 months. All our patients received induction treatment. The treatment was the 3+7 protocol, which consisted of daunorubicin at 45 mg/m^2 for 3 days + cytarabine at 100 mg/m^2 by continuous infusion for 7 days (the standard 3+7 induction protocol) [12, 13].

All our participant patients provided informed consent, and the study was approved by the Ethics Committee of Alexandria University, Faculty of Medicine.

All patients were subjected to full history taking, thorough clinical examination, and laboratory investigations, including the following: Peripheral blood sampling for CBC determination was performed on ADVIA 2120i automated blood cell counter (Siemens Healthcare Diagnostics, USA); BM aspiration and immunophenotyping were

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performed using Becton Dickinson, FACSCalibur flow cytometer equipped with Cell Quest software (USA) [14–16].

Immunophenotyping analysis

The detailed characterization of hematopoietic cells was done by analyzing the expression of a given set of antigens in a cell population. The applied panels of monoclonal antibodies (McAbs) for diagnosis of acute leukemia were as follows: primary panel: CD2 – phycoerythrin (PE); CD5 – PE; CD7 – fluorescein isothiocyanate (FITC); CD10 – FITC; CD19 – R-phycoerythrin (RPE); CD14 – FITC; CD13 – PE; CD33 – PE; HLA-Dr – FITC; CD34 – FITC; and CD45 – FITC; confirmatory antibodies: cytoplasmic (Cyt) CD22 – FITC; Cyt IgM – PE; Cyt CD3 – FITC; and Cyt anti-myeloperoxidase (MPO) – FITC).

For CD25 analysis, the stain/lyse/wash technique was applied. Briefly, 10 µl of the CD25-PE McAb (clone: TP1/6, cat. ref: 25PE-100T, HT-PE-0025-1, Immunostep, Spain) was added to 100 µl of ethylenediaminetetraacetic acid (EDTA) BM, mixed well, and incubated for 10 minutes at room temperature. The cells were then washed twice with phosphate-buffered saline (PBS); 2 ml lysing solution was added, mixed, and left for 10 minutes in the dark, and then the cells were washed twice with PBS. After the last wash, the cells were analyzed using a flow cytometer. During analysis, a gate was set around the required blast population. The cutoff point of positivity was considered when > 20% of the cells were stained with a particular antibody in excess of the background fluorescence in the negative controls. We used the cutoff value 20% according to the established flow cytometry protocol in the flow cytometry unit of our department.

Cytogenetic study

This study was done on 11 patients only because of the small sample size or bad metaphase quality, which was inadequate for karyotyping analysis in the remaining cases [17–19].

Chromosomes were prepared from dividing cells (mitotic cells).Cells were cultured in RPMI 1640 (cat. number: L0496-500, France), supplemented with fetal bovine serum (lot number: 0522D, Germany), L-glutamine (lot number: 1223D, Germany), as well as penicillin and streptomycin (cat. number: ECB3001D, Italy).

Cell division was halted at the metaphase, since, at this stage, the chromosomes condense and become recognizable as discrete units. This was achieved by destroying the cellular spindle using a metabolic inhibitor (colchicine).

The cells were treated with a hypotonic solution to encourage cell swelling and spreading of the chromosomes within the cells. They were then fixed and stained with Giemsa. This assists in chromosome banding (G-banding), and then metaphases were analyzed using the Cytovision Applied Imaging System (UK) with Olympus microscope CX41RF (S/N: 0L34790, Japan) equipped with camera ER3339 (S/N: 400561, Japan).

Data were fed into the computer and analyzed using IBM SPSS software package version 20.0 for statistical analysis.

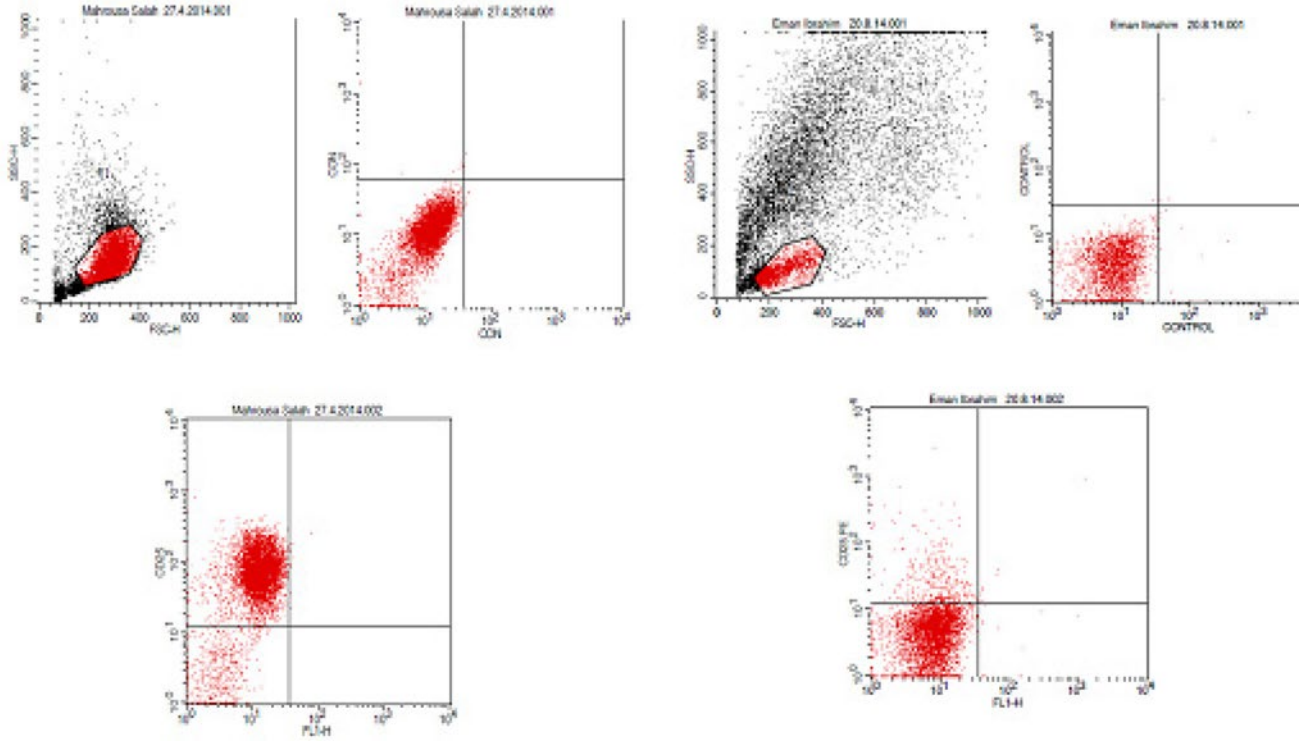

Fig. 1. Immunophenotyping of a CD25 positive case (left), and a negative case (right)

Results

Our group of AML patients consisted of 26 males (52%) and 24 females (48%). Their ages ranged from 14 to 82 years, with a mean of 41.2 years; only three patients were above 60 years of age. Demographic, clinical, and laboratory data are presented in table I. Our diagnosis was based on morphology, BM aspiration, and immunophenotyping. No other molecular markers were investigated in our study because of financial constraints.

Positive CD25 expression was found in 12 cases (24%) in our studied group (Fig. 1). Karyotyping was successfully done in 11 cases because of the inadequacy of the samples or bad quality of the metaphases in the remaining samples. According to the Medical Research Council (MRC) classification, eight cases were of intermediate risk having normal karyotypes, while the remaining three were of unfavorable risk. No favorable karyotype was detected.

The response of our patients was highly variable: 32 patients (64%) achieved CR from the first induction treatment and were doing well until the end of the study; three patients (6%) had a relapse after achieving CR; and 15 patients died (seven were nonresponders and died of acute respiratory distress syndrome (ARDS) and intracranial hemorrhage; three died during their first induction treatment from septicemia; and five died in the consolidation phase from sepsis or intracranial hemorrhage). None of our patients had BM transplantation.

On comparing CD25-positive and -negative patients in terms of the demographic data, clinical findings, CBC results, and karyotyping, no statistically significant difference was found (Tab. I, Fig. 2).

We tried to study the relation between CD25 expression and outcome and found that the CD25-positive group had significantly higher relapse and death rates ($P = 0.011$ and $P = 0.027$, respectively), apart from significantly lower remission rates ($P < 0.001$).

On combining the karyotype results with those of CD25 expression, CD25-positive AML patients with normal karyotype had a significantly lower remission rate and significantly higher relapse and death rates when compared with CD25-negative patients with normal karyotype ($P = 0.025$). Thus, CD25 added a significant prognostic value to the patients with intermediate cytogenetic risk (normal karyotype) (Tab. II).

Overall survival (OS) and leukemia-free survival (LFS) were studied in relation to age and CD25 expression. CD25-positive patients were associated with significantly worse OS ($P = 0.008$) (Fig. 3 A), shorter LFS ($P < 0.001$) (Fig. 3 B), and lower mean survival (7.9 months) in comparison with CD25-negative patients (14.4 months).

No significant differences between the age groups below and above 60 years in terms of OS($P = 0.084$) and LFS ($P = 0.130$) were found (Fig. 4).

We used the age, sex, white blood cell (WBC) count, hemoglobin concentration, platelet count, karyotype, percentage of blasts in the peripheral blood and BM, CD34 expression on the blast cells, and CD25 expression on the blast cells in our univariate analysis and then in the multivariate analysis against the outcome. In the univariate analysis, CD25 positivity was a strong predictor for bad outcome and worse prognosis ($P < 0.001$). In the multivariate analysis, CD25 positivity was the most powerful independent predictor for bad prognosis ($P = 0.002$).

Discussion

A variety of cytokines or ILs have been demonstrated to regulate the growth, survival, differentiation, and apoptosis of leukemic cells in vitro and in vivo [20, 21]. Cytokines bind to their respective receptors on the cell surface to exert their effect. However, little is known about the expression of cytokine receptors on the cell surface in AML and their clinical relevance [22].

A few researchers have suggested an unfavorable impact of CD25 expression in AML [10]. Furthermore, CD25-positive AML cells were shown to comprise a population of cell cycle-quiescent

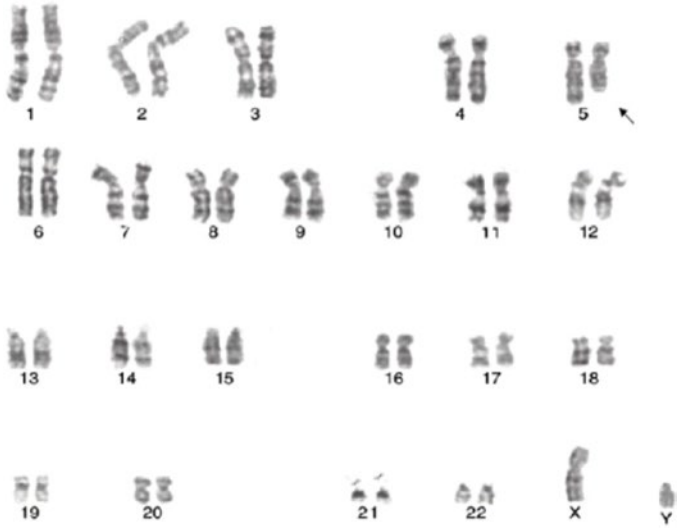

Fig. 2. Male karyotype showing a clone with a deletion of chromosome 5q (q31;q35)

Table I. Comparison between CD25-negative and -positive patients according to different demographic, clinical, and laboratory parameters

	Total (n = 50)	CD25		Test of significance	P
		Negative (n = 38)	Positive (n = 12)		
Sex					
Male	26 (52%)	17 (44.7%)	9 (75%)	$\chi^2 = 3.346$	0.067
Female	24 (48%)	21 (55.3%)	3 (25%)		
Age (years)	45 (14-82)	45 (14-75)	42 (20-82)	$U = 200.0$	0.524
< 60	47 (94%)	37 (97.4%)	10 (83.3%)	$\chi^2 = 3.185$	0.139
≥ 60	3 (6%)	1 (2.6%)	2 (16.7%)		
Fever					
No	30 (60%)	22 (57.9%)	8 (66.7%)	$\chi^2 = 0.292$	0.740
Present	20 (40%)	16 (42.1%)	4 (33.3%)		
Fatigue					
No	–	0 (0.0%)	0 (0.0%)	–	–
Present	50 (100%)	38 (100.0%)	12 (100.0%)		
Bony aches					
No	24 (48%)	21 (55.3%)	3 (25%)	$\chi^2 = 3.346$	0.067
Present	26 (52%)	17 (44.7%)	9 (75%)		
Pallor					
No	6 (12%)	4 (10.5%)	2 (16.7%)	$\chi^2 = 0.326$	0.621
Present	44 (88%)	34 (89.5%)	10 (83.3%)		
Bleeding					
No	38 (76%)	30 (78.9%)	8 (66.7%)	$\chi^2 = 0.754$	0.448
Present	12 (24%)	8 (21.1%)	4 (33.3%)		
Weight loss					
No	39 (78%)	29 (76.3%)	10 (83.3%)	$\chi^2 = 0.262$	1.000
Present	11 (22%)	9 (23.7%)	2 (16.7%)		
Lymphadenopathy					
No	48 (96%)	36 (94.7%)	12 (100%)	0.658	1.000
Present	2 (4%)	2 (5.3%)	0 (0.0%)		
Splenomegaly					
No	36 (72%)	27 (71.1%)	9 (75%)	$\chi^2 = 0.070$	1.000
Present	14 (28%)	11 (28.9%)	3 (25%)		
Hepatomegaly					
No	41 (82%)	31 (81.6%)	10 (83.3%)	$\chi^2 = 0.019$	1.000
Present	9 (18%)	7 (18.4%)	2 (16.7%)		
WBCs (x109/L)	43.9 (2-503)	43.9 (2-503)	52.5 (2-380)	$U = 195.50$	0.460
Platelets (x109/L)	36.5 (9-313)	36.5 (9-166)	35.5 (10-313)	$U = 204.50$	0.593
Hb (g/dl)	7.9 ± 1.5	7.7 ± 1.3	8.5 ± 1.7	$t = 1.655$	0.104
Peripheral blood blasts (%)	47 (8-92)	44.5 (8-90)	54 (28-92)	$U = 190.00$	0.388
BM aspirate blasts (%)	64 (22-98)	67 (22-98)	57.5 (29-92)	$U = 197.0$	0.481
FAB					
M0	1 (2%)	1 (2.6%)	0 (0.0%)	$\chi^2 = 5.740$	0.305
M1	5 (10%)	5 (13.2%)	0 (0.0%)		
M2	8 (16%)	5 (13.2%)	3 (25%)		
M4	23 (46%)	15 (39.5%)	8 (66.7%)		
M5a	12 (24%)	11 (28.9%)	1 (8.3%)		
M5b	1 (2%)	1 (2.6%)	0 (0.0%)		
CD34					
Negative	12 (24%)	10 (26.3%)	2 (16.7%)	$\chi^2 = 0.466$	0.705
Positive	38 (76%)	28 (73.7%)	10 (83.3%)		

	Total (n = 50)	CD25		Test of significance	P
		Negative (n = 38)	Positive (n = 12)		
Response					
Death	15 (30%)	8 (21.1%)	7 (58.3%)	$\chi^2 = 17.460^*$	$< 0.001^*$
Relapse	3 (6%)	0 (0.0%)	3 (25%)		
Remission	32 (64%)	30 (78.9%)	2 (16.7%)		
Prognosis					
Good	32 (64%)	30 (78.9%)	2 (16.7%)	$\chi^2 = 15.354^*$	$< 0.001^*$
Bad	18 (36%)	8 (21.1%)	10 (83.3%)		
Karyotyping: (MRC classification)					
Intermediate risk (normal)	8 (72.7%)	5 (62.5%)	3 (100%)	$\chi^2 = 1.547$	0.234
Unfavorable risk	3 (27.3%)	3 (37.5%)	0 (0.0%)		

χ^2, P: χ^2 and P values for chi-square test for comparing between the two groups.
U, p: U and P values for Mann–Whitney test for comparing between the two groups.
t, p: t and P values for Student's t-test for comparing between the two groups.
**: Statistically significant at P ≤ 0.05.*

Table II. Relation between karyotyping/CD25 and outcome

	Response			χ^2	P
	Death (n = 3)	Relapse (n = 1)	Remission (n = 7)		
Karyotyping/CD25					
Intermediate risk (normal) with CD25 +ve	2 (66.7%)	1 (100.0%)	0 (0.0%)	8.106^*	0.025^*
Intermediate risk (normal) with CD25 –ve	0 (0.0%)	0 (0.0%)	5 (71.4%)		
Unfavorable risk (abnormal) with CD25 +ve	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Unfavorable risk (abnormal) with CD25 –ve	1 (33.3%)	0 (0.0%)	2 (28.6%)		

χ^2, P: χ^2 and P values for chi-square test for comparing between the two groups.
**: Statistically significant at P ≤ 0.05.*

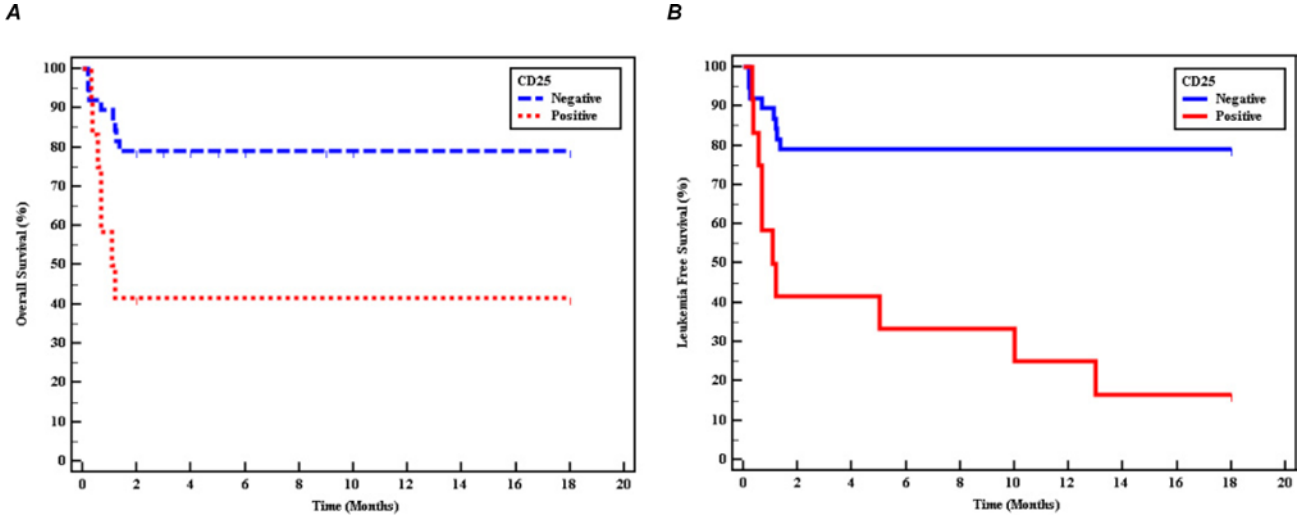

Fig. 3 A. Kaplan-Meier survival curve for overall survival with CD25; B. Kaplan-Meier survival curve for Leukemia-Free survival (LFS) with CD25

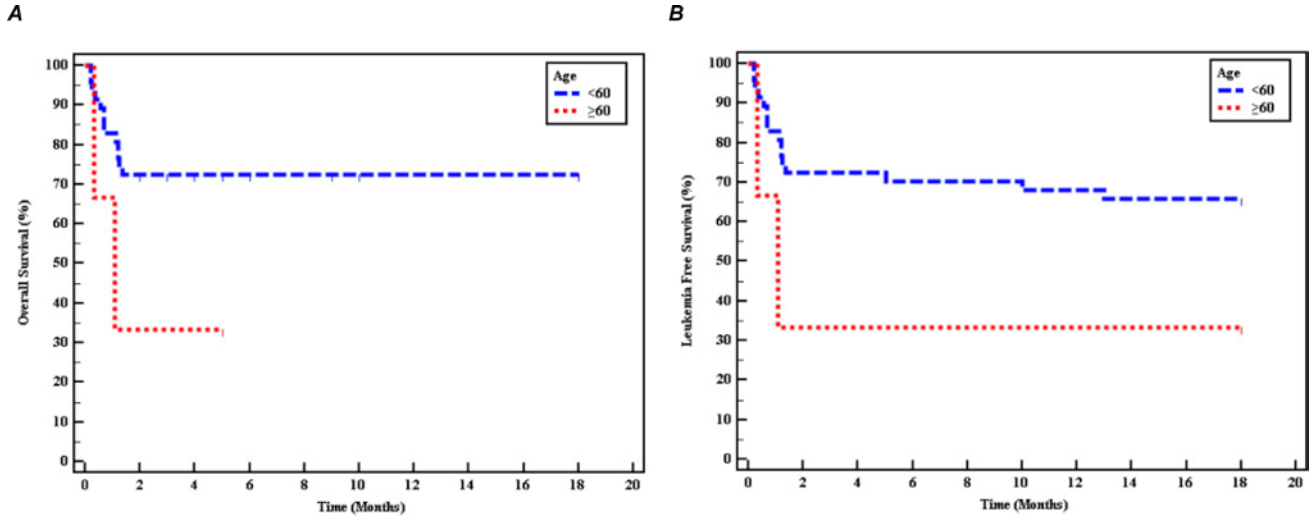

Fig. 4 A. Kaplan-Meier survival curve for overall survival with age; B. Kaplan-Meier survival curve for Leukemia-Free survival (LFS) with age

and chemotherapy-resistant leukemia stem cells (LSCs) [23], which requires newer therapeutic approaches targeting IL-2Ra to improve the prognosis of this type of AML.

Therefore, we aimed to evaluate CD25 expression in 50 newly diagnosed adult Egyptian patients with de novo AML, who were treated by the standard ''3+7'' protocol, correlate its expression with the biological characteristics of the disease and the clinical response, as well as assess its prognostic value, especially in the largest cytogenetic subclass of AML (those patients with a normal karyotype and patients with prognostically noninformative cytogenetic aberrations). We found that 24% of our patients had CD25-positive blasts at diagnosis.

Gonen et al [24] reported that 13% (87/657) of their AML patients had CD25-positive AML, while Fujiwara et al [25] demonstrated CD25-positive blasts in 14% (21/154) of de novo AML patients.

Gonen et al [24] and Nakase et al [26] demonstrated in their studies that CD25-positive AML patients presented with significantly higher WBCs ($P < 0.0001$ and $P = 0.00006$, respectively), which was not found in the current study ($P = 0.46$); similar to our findings, Terwijn et al [10] failed to find significant correlation between the percentage of CD25-positive blasts and WBC count at diagnosis ($P = 0.60$).

Cytogenetic analysis was done by Gonen et al [24] in all his AML patients, and the cytogenetic abnormalities were defined according to published criteria [27]. The study reported that distribution of cytogenetic risk classes differed significantly between CD25-positive and -negative patients ($P < 0.0001$); moreover, CD25-positive patients were mostly in the cytogenetically intermediate-risk group (92%). His findings were against ours, as we did not find any significant difference between both groups ($P = 0.234$) in having normal and abnormal karyotypes at presentation, which may be due to the small sample size of our study group and the fact that karyotyping was done in 11 cases only.

Similar to our findings, Cerny et al [28] did not detect any statistically significant difference in having normal and abnormal karyotypes and in the distribution of cytogenetic risk classes when comparing CD25-positive and -negative patients.

We investigated the effect of CD25 expression in AML patients on the clinical outcome; our results showed that CD25-positive AML patients with normal karyotype had significantly higher relapse and death rates and lower CR rate when compared with the CD25-negative patients with normal karyotype ($P = 0.025$). Moreover, CD25-positive patients were associated with worse OS and a significantly shorter LFS when compared to the CD25-negative patients.

Similarly, Terwijn et al [10] reported that CD25 expression on myeloblasts in AML patients was a valuable prognostic marker within the cytogenetically intermediate-risk group and CD25 positivity was associated with significantly decreased median OS of 10 months versus > 48 months in the CD25-negative group ($P = 0.0017$). The LFS in CD25-positive patients was 6 months versus > 47 months in the CD25-negative AML group. Our results were also in agreement with those of Cerny et al [28], Nakase et al [26], and Gönen et al [24], with Gönen et al [24] stating that CD25 expression was strongly associated with adverse outcome in the cytogenetically intermediate-risk patients.

From our results, we concluded that CD25 expression in intermediate-risk AML patients was significantly associated with adverse outcome. These data revealed that those patients had a greater likelihood of harboring unfavorable-risk mutations compared with CD25-negative cytogenetically intermediate-risk patients.

All studies, including the present study, consistently showed that the expression of CD25 in AML patients at presentation independently predicts poor prognosis. Moreover, some studies indicated target therapy for CD25-positive AML other than conventional chemotherapy to improve the prognosis of those patients.

Conclusions

Expression of CD25 in AML patients at presentation was associated with shorter OS and LFS.

CD25-positive AML patients who had normal karyotype at presentation showed lower remission rate and higher relapse and death rates when compared with patients who had abnormal karyotype.

Recommendations

Further studies need to be performed on CD25-positive AML patients on a wider scale to study other factors that might influence the results of chemotherapy treatment as well as to investigate other treatment strategies for CD25-positive AML.

Since the detection of CD25 is relatively straightforward and fast, we recommend that immunophenotypic detection of CD25 should be considered as a part of the routine workup of AML patients with normal karyotype, since it helps dissecting this heterogeneous cytogenetic category into prognostically different subgroups.

Acknowledgments

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Ethical approval

All procedures performed in our study were in accordance with the ethical standards of our institution and nation and align with the 1975 Helsinki declaration as revised in 2008.

Conflict of interest

The authors declare that they have no conflicts of interest.

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