Expression of programmed death ligand-1 (PDL-1) in Acute Myeloid Leukemia Patients and its relation to post induction Response

Abdel Hafeez LA[1], Mansor SG[1], Zahran AM[1], Hamed HB[1], Abdel Hameed MR[2], Bakry RM[1]

[1]Clinical Pathology Department, South Egypt Cancer Institute, Assiut University.
[2]Internal Medicine Department and Clinical Hematology Unit, Assiut University.

Corresponding Author: Lamiaa Ahmed Abdel Hafeez, Department of Clinical Pathology, South Egypt Cancer Institute, Assiut University, Assiut, Egypt. Email: r.d.lamia@hotmail.com

Abstract

Background and aim: In spite of approval of new therapeutic modalities, the clinical outcome of patients with acute myeloid leukemia (AML) remains unsatisfactory. Accurate risk stratification of patients is crucial for improving their outcome. Programmed death receptor-ligand 1 (PD-L1) plays an important role in the progression of malignant disorders. So we aimed to analyze PD-L1 expression on AML cells by flow cytometry and its relation to post induction response.

Patients and Methods:

Our study was conducted on fifty three newly diagnosed AML patients presented to pediatric and medical oncology departments in south Egypt cancer institute. Age ranged from 1 year to 74 years old with median age 26 years. Assessment of PD-L1 expression on blasts was performed by flow cytometry and its relation to post induction therapy according based on complete blood counts and BM blast percent .Student t-test and spearman's correlation were used to determine significant relations and correlations. An informed written consent was taken from all cases.

Results:

The study showed PD-L1 mean expression percentage by flow cytometry of 18.69±3.35. PD-L1 expression significantly affected the response to induction therapy so that higher expression was associated with poor response to induction therapy.

Keywords:
Acute myeloid leukemia, PD-L1

Introduction:

AML is a hematological neoplasm characterized by accumulation of malignant poorly differentiated immature myeloid cells within the bone marrow, peripheral blood and extra medullary infiltration [1]. AML is the most common acute leukemia in adults. Although advances in the treatment of AML have led to significant improvements in outcomes for younger patients, prognosis in the elderly who account for the majority of new cases remains suboptimal [2]. Leukemic cells apply multiple immune evasion mechanisms resulting in tumor progression. One of the most important immune escape mechanisms is over-expression of immune checkpoint receptors and their ligands such as PD-1 and PD-L1 [3].

PD-1/PD-L1 pathway controls the induction and maintenance of immune tolerance within the tumor microenvironment. The activity of PD-1 and its ligands PD-L1 or PD-L2 are responsible for T cell activation, proliferation, and cytotoxic secretion in cancer to produce anti-tumor immune responses [4]. This study aim to evaluate the expression of PDL-1 in acute myeloid leukemia and it's relation to post induction response.

Patients and Methods:

This prospective study; included fifty three newly diagnosed AML patients presented to South Egypt cancer institute (SECI), Assiut University, the study was held between June 2020 to February 2021

Inclusion criteria

(1) Age range from 1 to 74 years old.
(2) Patients with de-novo AML.

Exclusion criteria

(1) Relapsed patients with AML.
(2) Patients with AML who started chemotherapy before enrolment in the study.
(3) Secondary AML with preceding hematologic disorder or solid tumors.
(4) Other types of acute leukemia other than AML.

(5) Past history of autoimmunity.
(6) Age less than 1 year.

Methods

All patients were subjected to history taking and clinical examination, with careful assessment of clinical signs relevant to leukemia as hepatomegaly, splenomegaly, lymphadenopathy, and gum or skin infiltration. Complete blood pictures were performed by the fully automated blood counter (CD Ruby). The patients were subjected to Flow cytometric immunophenotyping using monoclonal antibodies that were used for diagnosing AML including: CD34, CD45, CD19, CD3, CD5, CD7, CD13, CD33, CD117, CD15 and intracellular myeloperoxidase, CD14, HLA-DR, CD41, CD61 and anti-glycophorin A. All monoclonal antibodies were purchased from Becton Dickinson (BD) Bioscience, CA, USA – Flow cytometric detection of PD-L1 expression on myeloid blasts. The diagnosis was based on standard morphologic, immunophenotypic, and cytogenetic criteria. Conventional induction therapy with 3 days of an anthracycline and 7 days of cytarabine ("3 + 7"), response assessment is commonly performed between day 21 and day 28 after start of therapy**.**

Response to treatment was defined according to the revised recommendations of the International Working Group for Diagnosis, the Standardization of Response Criteria, the Treatment Outcomes, and the Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.

Patients were evaluated for the response after induction therapy clinically and by complete blood picture and bone marrow aspirate examination.

- Remission cases in which they are clinically free, normal CBC and normal BMA after induction phase (blast cells less than 5%).
- Non remission cases include: non responding cases in which (clinically show persistent hepatosplenomegaly or lymphadenopathy, persistent cytopenia and BMA shows blast cell 5% or more) including cases died during or after finishing induction phase [5].

Flow cytometric detection of PD-L1

Fifty µl of bone marrow sample was incubated with 5 µl of PE- anti- PD-L1 (from BD Biosciences, CA, USA) for 20 minutes at room temperature in the dark. Following incubation, red blood cells lysis and washing with PBS were done. After washing, the cells were re-suspended in PBS, and analyzed by BD FACSCanto cell analyzer with face diva software. An isotype matched negative control was used with samples. Forward and side scatter histogram was used to define the blast cell population after final diagnosis of AML, then the expression of PD-L1 was detected on myeloid blasts.

Follow up of patients was done after 28 days to detect morphological remission rate (bone marrow blast percent less than 5 %) after first induction therapy.

Statistical Analysis:

Data analysis was done by using The Statistical Package for Social Science (SPSS) version 21 (IBM, New York, USA). Continuous data was presented as mean, median, standard error of mean and range. Categorical data was presented as frequency and percentage. For quantitative data, comparison between two groups was done using Student t-test and Kruskall Wallis were used for comparison between groups. Correlations were calculated by the Spearman correlation coefficient.

Results:

Demographic and laboratory characteristics of the studied AML patients at presentation are displayed in Table 1, of total fifty three patients, there were 29 males and 24 females with a median age of 26 years and ranging from 1 to 74 years.

Regarding hematological data of the studied patients at presentation, the mean WBC count was 62.40 ±9.57 $x10^3$/mm3 while the mean hemoglobin (Hb) concentration was 8.81±0.28 g/dL and the mean platelet count was 69.32 ± 10.16 $x10^3/mm^3$, Bone marrow studies at the time of diagnosis showed mean blast count of 58.06±3.28 %.

The most common subtype of AML presented in the study group according to FAB classification was AML M 5 that was seen in 14 (26.4 %) cases followed by AML M 4 and M2 each in 12 (22.6 %) then AML M3 in 6 (11.3 %), then AML M 0 in 5 (9.4 %) followed by AML M7 and M 1 both seen in 2 patients (3.8 %) (Table 2).

There was no statistical significant difference between FAB subtypes of AML and PDL-1 expression (p= 0.072) (Table 3).

The mean value of PDL1 expression percentage was 18.69±3.35 with range 1.04 - 79.56.

On follow up of patients after they received induction therapy, we found that 36 (67.9%) patients showed remission with mean PD-L1 expression of 9.70±2.36, while 17 (32.1 %) patients showed poor outcome after induction therapy with mean PD-L1 expression of 37.71±7.38 (Table 3).

Comparison of the laboratory data of the patients that achieved remission and those that not achieved remission, revealed that the mean values of PDL1 expression and percent of bone marrow blasts were significantly lower in patients who achieved remission than those who did not achieve remission (p=0.002 and p =0.001) respectively (Table 4).

On correlation of PDL-1 expression with various patients' laboratory data, there was significant positive correlation with the percent of bone marrow blasts (r=0.404, p=0.014), There were no statistically significant correlations between PDL-1 with HB, WBCs or platelets (Table 5).

Table (1): Basic Demographic and laboratory characteristics of the studied AML patients at presentation:

Variable	Total (n=53)
Age (years)	
Median	26
Range	(1- 74)
Sex (n %)	
Female	24 (45.3%)
Male	29 (54.7%)
HB (g/dL)	
Mean ± SEM	8.81±0.28
Range	(4.2 – 14.1)
WBCs ($x10^{3}/mm^{3}$)	
Mean ± SEM	62.40 ±9.57
Range	(0.5 – 310)
Platelets ($x10^{3}/mm^{3}$)	
Mean ± SEM	69.32 ± 10.16
Range	(4 - 348)
Bone marrow blasts (%)	
Mean ± SEM	58.06±3.28
Range	(20 - 95)
Bone marrow cellularity	
Normocellular	19 (35.8%)
Hypocellular	12 (22.6%)
Hypercellular	22 (41.5%)

SEM: standard error of mean, Hb; hemoglobin; WBC, white blood cell.

Table (2) Distribution of studied patients based on FAB classification of AML

Parameters		Study group (n= 53)	
		n	%
FAB subtypes	**AML M0**	5	9.4 %
	AML M1	2	3.8 %
	AML M2	12	22.6 %
	AML M3	6	11.3 %
	AML M4	12	22.6 %
	AML M5	14	26.4 %
	AML M7	2	3.8 %

FAB: French-American-British

Table (3) Comparison among different acute myeloid leukemia fab subtypes in terms of their PD-L1 expression

	FAB subtypes	Mean	SEM	median	Test	*p*-value
PD-L1 expression by flow cytometry	AML M0	26.87	12.91	14.69	11.595	0.072
	AML M1	6.38	.78	6.38		
	AML M2	9.23	5.81	3.34		
	AML M3	7.84	4.28	1.82		
	AML M4	33.19	8.05	27.55		
	AML M5	19.63	6.96	4.81		
	AML M7	6.31	2.01	6.31		

PD-L1: Programmed death ligand-1, SEM: Standard error of mean

Table (4) Comparison between laboratory data of the studied AML patients according to response to induction therapy

	Remission	Non remission	*p*-value
Total number of patients	n=36 (67.9%)	n=17 (32.1 %)	
PDL-1 surface expression percentage	9.70±2.36	37.71±7.38	**0.002**
HB (g/dl)	8.93±0.37	8.54±0.43	0.532
WBCs ($\times10^{3}/mm^{3}$)	54.58±10.00	78.98±20.97	0.238
Platelets ($\times10^{3}/mm^{3}$)	65.86±11.41	76.65±20.95	0.325
Bone marrow blast percent (%)	49.33±3.71	76.53±3.73	**0.001**

* Student t test was used, Hb; hemoglobin; PDL-1, programmed death ligand 1; WBC, white blood cell.

Table (5) Correlations between laboratory data and percent of PD-L1 expression on myeloid blast cells studied AML patients of by flow cytometry

Laboratory data	PDL-1 expression
WBC ($\times10^{3}/mm^{3}$)	
r	0.119
p	0.398
Hb (g/dl)	
r	-0.198
p	0.155
Platelet ($\times10^{3}/mm^{3}$)	
r	-0.076
p	0.587
Bone marrow blasts (%)	
r	0.337
p	0.014

* Test done by Spearman's correlation, statistically significant correlation ($p<0.05$) Hb; hemoglobin; PDL-1, programmed death ligand 1; WBC, white blood cell, p; p value, r; correlation coefficient.

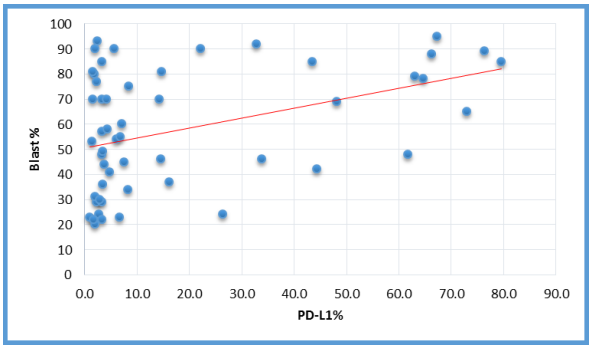
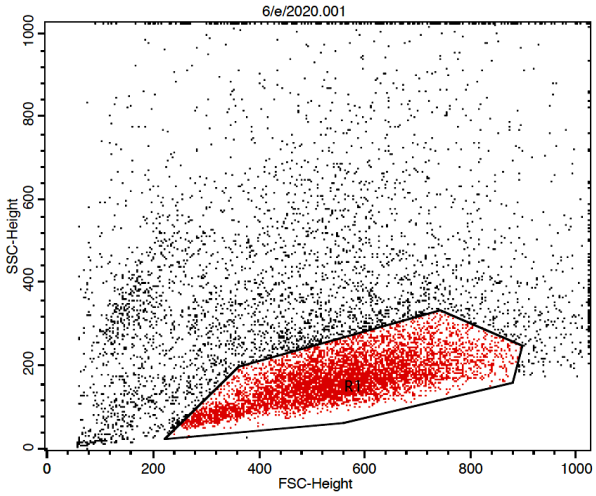


Figure (1): Scatter diagram showing correlations between blasts and PDL1 expression in AML patients.



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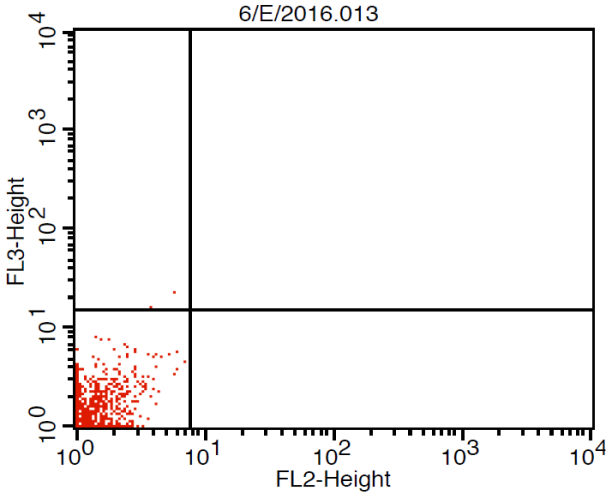
Region	Events	% Gated
R1	6076	60.76

a



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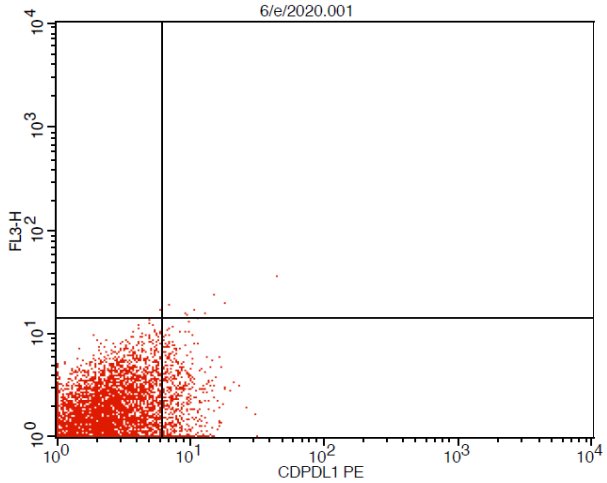

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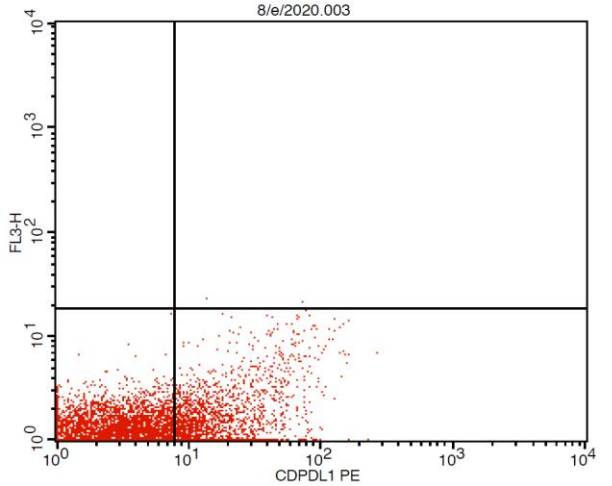
Quad	Events	% Gated
UL	1	0.02
UR	9	0.15
LL	5537	91.13
LR	529	8.71

c



File: 8/e/2020.003
Gate: G1

Quad	Events	% Gated
UL	0	0.00
UR	2	0.03
LL	5883	73.81
LR	2085	26.16

d

Figure (2): a: forward and side scatter of defined Gated cells, b: auto control, c: myeloid blast cells with low expression of PD-L1, d: myeloid blast cells with high expression of PD-L1

Discussion:

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults and has an incidence of 4.3 per 100,000 populations. The incidence of AML increases significantly with age with an age range at diagnosis of 65–72 years [6].

Previous studies suggest that pathogenesis of AML is related to highly immunosuppressive tumor microenvironment that is mediated through regulatory T (Treg) cells, the interaction between AML cells and Treg cells is mediated by PD-L1 [7].

PD-L1 belongs to the B7 series and it is type 1 trans membrane glycoprotein which is expressed by macrophages, some activated T cells and B cells and some epithelial cells, In addition, PD-L1 is expressed by tumor cells as an "adaptive immune mechanism" to escape anti-tumor responses by down regulating effector T cell activity [4].

The main aim of our study is to identify the relationship between PD-L1 expression in patients with denovo AML and different disease characteristics and the clinical response to induction chemotherapy.

Our study showed PD-L1 expression mean value of 18.69 ranged from 1% to 79.6%, which is near to results reported by mostafa et al who demonstrated that PD-L1 expression in their patients ranged from 1.52% to 88.1% [5].

There was no significant correlation between PD-L1 expression with various laboratory findings including WBCs, Hb and platelet count, these results are concomitant with results found by Berthon et al, who included 79 patients in their study and revealed no correlation between PD-L1 expression and different lab characteristics [7].

We found a significant positive correlation between PD-L1 expression with BM blast cells (r=0.337, p=0.014). This finding was not in line with Mostafa et al., who demonstrated no significant correlation with bone marrow blast percentage, this different result may be attributed to the difference in total patient number as their study was conducted on 40 adult patients while ours included 53 patients [5].

Regarding the association between PD-L1 expression and outcome after induction therapy we found that higher percentage of remission rate was significantly related to low PD-L1 expression (p=0.002) and this agree with Zhang et al., who showed that complete remission (CR) rate was lower in PD-L1 positive cases; (66.7% in +ve group vs. 71.4% in -ve group). Also, the relapse rate and the proportion of refractory patients in PD-L1 positive group were higher than those in the PD-L1 negative group [8].

Zajac et al. showed that PD-L1 expression is associated with unfavourable clinical outcome in AML patients [9]. Also Annibali et al. concluded that the appearance of PD-L1 on AML blasts was associated with the negative course of the disease [10].

Conclusion:

Our data suggest that PD-L1 is expressed in AML cases with variable degrees. PD-L1 expression level is correlated to bone marrow blast percent, we support that PD-L1 expression level affect the outcome after induction therapy in AML patients.

List of Abbreviations:

PD-L1: Programmed death ligand-1
AML: Acute Myeloid Leukemia
FAB: French American British

Competing interests: The authors report no conflicts of interest associated with this work

Authors' Contributions:

Dr. Lamiaa Ahmed has carried out the collection of samples as well as analysis and interpretation of results and drafted the manuscript.

Dr. Asmaa Mohamed Zahran and Dr.Shima Gafar Mansor have contributed to designing the work

Dr. Hosni badrawy Hamed and Dr.Muhamed Ramadan have contributed by revising the work.

Dr. Rania M Bakry has contributed to supervising and revising the work.

All Authors have read and approved the final manuscript.

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