Original Article

Research in Oncology
2020; Vol. 16, No. 1: 6-10.
DOI: 10.21608/resoncol.2020.24758.1091

Prognostic Value of CD56 Expression in Multiple Myeloma

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Background: Understanding the prognostic markers of multiple myeloma (MM) helps in optimizing therapeutic approaches. CD56 is frequently expressed by malignant plasma cells and its use as a prognostic marker in MM is promising.
Aim: To evaluate prognostic value of CD56 expression in patients with MM.
Methods: This study included 50 newly diagnosed patients with MM. Bone marrow samples were analyzed for CD56 expression by flow cytometry. All patients received bortezomib-based therapy for at least 3-4 months.
Results: The median age of patients was 52 years (range 32-75) and 54% of them were males. The stage according to the International Staging System was I in 15 (30%) patients, II in 18 (36%) and III in 17 (34%). CD56 positivity was detected in 84% of enrolled patients. Multivariate analysis revealed that the lack of CD56 expression was an independent predictor of worse overall survival (HR = 4.31 [95% Confidence Interval: 1.23 – 15.13], p = 0.002).
Conclusion: The present study suggests that CD56 negativity is associated with poor prognosis in patients with MM and that its incorporation in the risk panel of MM may be considered. Further studies with larger sample size to validate its prognostic value are needed.

Keywords: Multiple myeloma, Flow cytometry, CD56, Prognosis.
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Submitted: 26-February-2020, **Revised**: 15-March-2020, **Accepted**: 21-March-2020, **Published online**: 28-March-2020

INTRODUCTION

Multiple myeloma (MM) is a B-cell clonal disease that causes accumulation of malignant plasma cells (PCs) in the bone marrow. This accumulation leads to bony lesions as well as increased levels of serum and urinary monoclonal proteins. Patients with MM may develop hypercalcemia, anemia or renal insufficiency [1].

Flow cytometric immunophenotyping is important in MM for the diagnosis, predicting prognosis and monitoring treatment [2-4]. Multiparameter flow cytometry (MFC) is a valid method in the fast recognition of clonality criterion of the cells and their aberrant expression of antigens [5]. For a case of MM, the required antibodies panel reported by the European Myeloma Network is CD38, CD138, CD19, CD45, CD56, CD20, CD117, CD28, and CD27. It recommends a minimum of five initial gating parameters (CD38, CD138, CD45, FSC, and SSC properties) within the same tube for the calculation of total plasma cells [6].

CD56 (neural cell adhesion molecule) is a membrane glycoprotein which is expressed frequently by malignant plasma cells, but not normal plasma cells [7-8]. In recent years, some studies assessed the relationship between MM prognosis and the expression of CD56 [9-11]. Pan et al retrospectively assessed the importance of CD56 as a prognostic factor in 50 newly diagnosed MM patients [11]. They found that patients with CD56 expression had a better overall response rate (p = 0.024). In addition, in multivariate analysis, CD56 positivity was associated independently with longer overall survival (OS) (p = 0.012) [11].

In this study, we examined the relationship between CD56 expression by malignant plasma cells in newly diagnosed MM patients and clinical as well as laboratory parameters to determine its prognostic value.

METHODS

This retrospective study included 50 patients with MM who had been treated during an 8-year period (from January 2010 to December 2017) at the Medical Oncology Department, South Egypt Cancer Institute. The study was approved by the Ethical Committee, Assiut University.

The International Myeloma Working Group (IMWG) [12] criteria was used for the diagnosis of MM and the Revised International Staging System (R-ISS) [13] for its staging. All patients received bortezomib-based therapy that included bortezomib/cyclophosphamide/ dexamethasone for at least 3 months.

Complete blood picture (using Cell-Dyn 3500C S, Abbott Diagnostics, USA), serum chemistry including creatinine, calcium, albumin and β2 microglobulin (using COBAS Integra 400 Plus, Roche, Switzerland) and serum protein electrophoresis (using Pretty Interlab, Interlab,

Italy) were performed for all patients. Bone marrow aspiration (BMA) and biopsy (BMB) and immunophenotyping data were also obtained. Immunophenotyping was performed with a panel of monoclonal antibodies: anti-CD38-fluorescence Isothiocyanate (FITC) conjugated, anti-CD56-allophycocyanin (APC) conjugated (Beckman Coulter, Germany), anti-CD138-phycoerythtin (PE) conjugated, anti-CD19- peridinin chlorophyll protein (PerCP) conjugated, anti-Kappa light chain-FITC conjugated and anti-Lambda light chain-PE conjugated (BD Bioscience, USA).

The Statistical Package for Social Science (SPSS) version 21 (IBM, New York, USA) was used for storing, manipulation, interpretation of collected data. Continuous data was presented as mean and standard deviation (SD) or median and range, while categorical data was presented as frequency and percentage. *Chi*²-test and ANOVA tests were used to calculate the relation between the nominal data of two different groups and more than two groups respectively. Overall survival was calculated from the date of diagnosis to the date of death. Censored patients were those alive at last encounter, whether still under regular follow up or lost to follow up. Kaplan-Meier method was used to estimate survival and Log-rank test to identify the significance of the difference in survival between groups in univariate analysis. We examined the prognostic factors in the multivariable analysis using Cox proportional hazards model to test their independent significance. P-value less than 0.05 was considered significant.

RESULTS

The demographics and baseline characteristics of the enrolled 50 patients are shown in Table 1.

In all cases, plasma cells could be sufficiently identified through CD138 positive gating on mononuclear cells. CD56 was positive in the majority (84%) of patients.

Table 2 shows a comparison between CD56 positive and CD56 negative patients. There was no significant difference in the studied variables according to CD56 expression status.

After a median follow up period of 24.5 months (10-84 months), the median OS of all patients was 30 months (95% CI 24.54 - 35.46) as shown in Figure 1.

Univariate analysis of overall survival is illustrated in table 3. Overall survival was significantly shorter in patients with negative CD56 disease compared to those with positive CD56 (Figure 2). In addition, advanced stage, renal impairment and higher B2 microglobulin level were associated with significantly shorter OS (Table 3). The remaining studied factors did not show a significant correlation.

In multivariate analysis, negative CD56 expression was an independent predictor of worse OS (Table 4). Renal impairment was independently associated with worse OS as well.

Table 1: Demographics and baseline characteristics of 50 patients with multiple myeloma

Characteristic		
	Median	**Range**
Age (years)	52	32-75
	No.	**%**
Sex		
Males	27	54
Females	23	46
Stage		
I	15	30
II	18	36
III	17	34
Anemia		
yes	28	56
No	22	44
Hypercalcemia		
yes	21	42
No	29	58
Renal impairment		
yes	13	26
No	37	74
Albumin (gm/L)		
≥35	18	36
< 35	32	64
B2 microglobulin (mg/L)		
≥3.5	36	72
< 3.5	14	28
CD56 expression		
Positive	42	84
Negative	8	16
	Mean	**SD**
White blood cells count (10^9/L)	8.28	4.46
Platelets count (10^9/L)	218.44	83.41
Hemoglobin level (gm/dL)	9.72	1.66
BMA plasma cell (%)	29.50	19.13
BMB plasma cell (%)	52.12	20.6

BMA: Bone marrow aspirate; **BMB**: Bone marrow biopsy; **SD**: Standard deviation

DISCUSSION

CD56 is a homophilic binding glycoprotein implicated in cell-cell adhesion that is involved in attaching plasma cells to the stromal structure of the bone marrow [1]. The prevalence of CD56 positivity in MM varied among studies [11, 14-16]. In the current study, 84% of patients were CD56 positive, which is much higher than the 42% prevalence found by Boshnak and Hashem [14]. Other studies done by Pan et al [11], Qiu et al [15] and Shim et al [16] reported a prevalence of 71%, 61% and 66%; which is not much lower than that in our study.

Table 2: Relation between CD56 expression status and baseline characteristics

Characteristic	Positive CD56 (n=42)	Negative CD56 (n=8)	p-value
	No. (%)	No. (%)	
Age (years)			
<65	37 (88.1)	6 (75)	0.31
≥ 65	5 (11.9)	2 (25)	
Sex			
Male	23 (54.8)	4 (50)	0.552
Females	19 (45.2)	4 (50)	
Stage			
I/II	28 (66.7)	5 (62.5)	0.558
III	14 (33.3)	3 (37.5)	
Anemia			
Yes	23 (54.8)	5 (62.5)	0.498
No	19 (45.2)	3 (37.5)	
Thrombocytopenia			
Yes	33 (78.6)	5 (62.5)	0.287
No	9 (21.4)	3 (37.5)	
Hypercalcemia			
Yes	17 (40.5)	4 (50)	0.451
No	25 (59.5)	4 (50)	
Renal impairment			
Yes	11 (26.2)	2 (25)	0.659
No	31 (73.8)	6 (75)	
Hypoalbuminemia			
Yes	15 (35.7)	3 (37.5)	0.609
No	27 (64.3)	5 (62.5)	
B2 microglobulin (mg/L)			
≥3.5	30 (71.4)	6 (75)	0.604
< 3.5	12 (28.6)	2 (25)	
Bony lesions			
Yes	31 (73.8)	6 (75)	0.115
No	11 (26.2)	2 (25)	

Table 3: Univariate analysis of overall survival in 50 patients with multiple myeloma

Variable	Median OS (months)	95% CI	p-value
Age (years)			
≥ 65	30	17.58 - 42.41	0.501
< 65	28	23.97 - 32.03	
Sex			
Male	25	17.07 - 32.92	0.152
Female	30	26.42 - 33.57	
Stage			
I	36	31.89 - 40.1	0.019
II	28	23.51 - 32.48	
III	25	20.46 - 29.53	
Anemia			
Yes	30	23.82 - 36.17	0.211
No	30	20.22 - 39-77	
Hypercalcemia			
Yes	35	18.71 - 51.28	0.712
No	28	23.6 - 32.29	
Renal impairment			
Yes	25	23.39 - 26.6	0.008
No	33	27.7 - 38.29	
Albumin level (gm/L)			
≥35	33	24.4 - 41.59	0.215
< 35	25	20.2 - 29.79	
B2 microglobulin (mg/L)			
≥3.5	25	23.76 - 26.24	0.032
< 3.5	36	31.67 - 40.32	
CD56 expression			
Positive	30	27.36 - 32.64	0.006
Negative	24	10.14 - 37.85	

CI: Confidence interval, **OS**: Overall survival

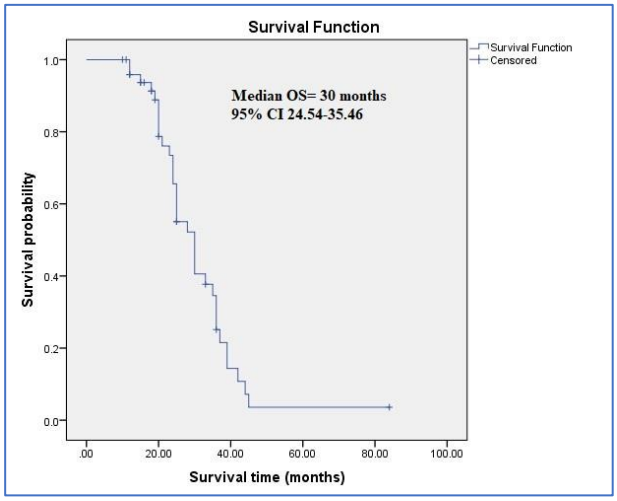

Figure 1: Kaplan Meier curve showing overall survival of all patients

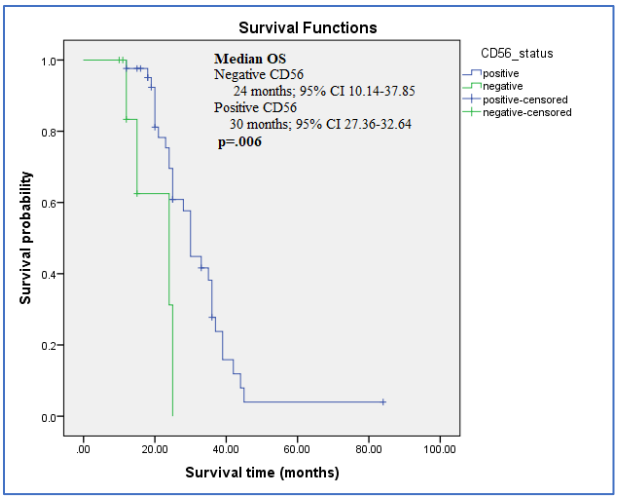

Figure 2: Kaplan Meier curves showing overall survival according to CD56 expression status

Table 4: Multivariate analysis of overall survival in 50 patients with multiple myeloma

Variable	HR	95% CI	p-value
Advanced stage	1.55	0.77 - 3.11	0.217
No renal impairment	0.30	0.12 - 0.78	0.014
Higher B2 microglobulin	1.05	0.28 - 3.93	0.936
Negative CD56 expression	4.31	1.23 - 15.13	0.002

HR: Hazard ratio, **CI**: Confidence interval

The presence of CD56 has been related to malignancy in plasma cells, and its downregulation has been shown to be associated with high proliferation and spreading of malignant plasma cells [9]. In this study, we found that CD56 expression had no negative effect on the prevalence of clinical manifestations.

In contrast to our results, Ngo et al stated that the presence of CD56 correlates with the aggressiveness of disease in myeloma patients [10]. Also, Pan et al found that CD56 negativity is associated with a lower frequency of bony lesions, which differs from our finding that CD56 expression status is not associated with a significant change in the incidence of bony lesions.

Unlike the results of our study, Boshnak and Hashem found that CD56 positivity was associated with advanced stage [14]. However, the results of Ceran et al [17] agree with our results, as CD56 expression was found to have no effect on clinical behavior. Such discrepancies may be attributed to differences in the sample size.

The lack of expression of CD56 was found to be an independent poor prognostic factor, which confirms the findings of other studies [11, 15, 18]. Overall survival was significantly reduced in patients having negative CD56 disease when compared to those with positive CD56. This finding is consistent with the findings of Skerget et al who found that lack of CD56 expression is a bad prognostic factor in patients with MM even with bortezomib induction [18]. Of note, they estimated the prognostic value of CD56 expression in relation to progression-free survival, not overall survival as in our study. Also, our results agree with that reported by Qiu et al who that the OS of CD56 positive patients is significantly better than that of CD56 negative patients (53 months vs 31 months, respectively; $p = 0.016$) [15].

The limitations of our study include the small sample size, being a single center one and its retrospective design. In addition, we did not include other known prognostic factors in MM, like lactate dehydrogenase (LDH) serum level.

Conclusion

The present study suggests that CD56 negativity is associated with poor prognosis in patients with MM, independently of other factors.

The incorporation of CD56 in risk stratification of MM patients may be considered.

Further prospective studies with larger sample are needed to validate its prognostic role.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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