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Prognostic significance of CD56 antigen in newly diagnosed multiple myeloma

A real-world retrospective study

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Abstract

The prognostic value of plasma cell CD56 expression of patients with multiple myeloma (MM) has been reported in many studies, but the results are controversial. This study aimed to examine the prognostic significance of CD56 in MM patients.

Eighty seven patients with newly diagnosed MM were enrolled in this study, and their clinical characteristics, immunophenotypes, and cytogenetics were retrospectively analyzed to explore the prognostic significance of CD56 expression. Multiparameter flow cytometry was used to detect MM in bone marrow samples from all patients. Patients were divided into 2 groups based on whether they expressed CD56: CD56 + group and CD56 – group.

After 4 cycles of chemotherapy, the overall response rate of the CD56 – patients was lower than that of the CD56 + patients (60.0% vs 81.1%, $P = .036$). Survival analysis showed that the median progression-free survival (PFS) was 10 months for the CD56 – group and 27 months for the CD56 + group ($P = .007$). The median overall survival (OS) of patients for the CD56 – group was 25 months versus not reached in the CD56 + group ($P = .010$). In addition, among the high-risk patients detected by fluorescence in situ hybridization (FISH), the median PFS was 4 months for the CD56 – group and 16 months for the CD56 + group ($P = .012$). The median OS of the CD56 + group and CD56 – group was 36 months and 15 months, respectively, with statistically significant differences ($P = .017$).

Our study confirmed that CD56 – patients with MM had a worse prognosis than that of CD56 + patients with MM. Among the patients with ≥ 2 high-risk cytogenetics, the existence of the CD56 negativity can further identify MM patients with poor PFS and OS.

Abbreviations: aHSCT = autologous hematopoietic stem cell transplantation, ALB = albumin; β2-MG = β2-microglobulin, BMPCs = bone marrow plasma cells, Ca = calcium, CR = complete response, eGFR = estimated glomerular filtration rate, FISH = fluorescence in situ hybridization, HGB = hemoglobin, HRCAs = high-risk cytogenetic abnormalities, LDH = lactate dehydrogenase, MFC = multiparameter flow cytometry, MM = multiple myeloma, OS = overall survival, PCs = plasma cells, PFS = progression-free survival, PLT = platelet, VGPR = very good partial response.

Keywords: CD56, cytogenetic, immunophenotype, multiple myeloma, survival

1. Introduction

Multiple myeloma (MM) is one of the most common proliferative malignant tumors of the hematological system, more precisely of plasma cells (PCs). It usually occurs in middle-aged and elderly people, accounting for approximately 1% of all malignant tumors and 10 to 15% of hematological tumors.[1] MM is characterized by the expansion of clonal PCs in the bone marrow, which produces monoclonal immunoglobulins, leading to anemia, bone destruction, hypercalcemia, or renal insufficiency,[2] among other conditions. Multiparameter flow cytometry (MFC) has become a vital basis for the clinical diagnosis and classification of MM because of its rapid, accurate, and objective detection of the biological characteristics of normal and neoplastic MM cells. Studies have shown that the expression of plasma cell surface antigens is associated with the prognosis of MM.[3–5] CD56, a membrane glycoprotein of the immunoglobulin superfamily, is involved in cell growth and migration as a nerve cell adhesion molecule (NCAM),[6,7] and 70 to 80% of MM patients are CD56-positive (CD56+).[8] CD56 expression in MM correlates with different clinicopathological behaviors. CD56 absence leads to increased secretion of MMP-9, which promotes basement membrane degradation, invasion, and metastasis of myeloma cells,[9,10] suggesting that patients

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

The study protocol was approved by the institutional review board of The Second Hospital of Jilin University. Due to the retrospective design of the study, the local ethic committee confirmed that informed consent was not necessary from participants. The demand of patient informed consent was deserted because of the retrospective nature of this study.

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with CD56-negative (CD56−) may have a worse prognosis. The prognostic value of plasma cell CD56 expression in patients with MM has been reported in several studies,[3,11–15] but the results are controversial. Using conventional treatments, Sahara et al[12] showed that CD56 − patients had significantly lower survival than that of CD56 + patients. In contrast, Hundemer et al[16] showed that absence of CD56 was not a marker of bad outcome in patients with MM administered high doses of chemotherapy. Several recent studies revealed that novel drug therapies (such as bortezomib or lenalidomide) cannot overcome the negative effects of the lack of CD56 expression.[14,17,18] In addition, according to a recent meta-analysis,[19] CD56 negativity is a poor prognostic factor for overall survival (OS) and progression-free survival (PFS) in MM patients. To further explore the prognostic significance of CD56 expression in MM patients, we retrospectively analyzed the clinical, immunophenotypic, and cytogenetic characteristics of 87 newly diagnosed MM patients.

2. Materials and Methods

2.1. Patient characteristics

A retrospective analysis was performed on 87 newly diagnosed MM patients admitted to the Second Hospital of Jilin University between January 2016 and April 2021. The 46 male and 41 female enrolled, presented a median age of 62 (35–88) years. All patients met the 2014 International Myeloma Working Group (IMWG) diagnostic criteria.[20] The MM clinical stages were based on the International Staging System (ISS) risk stratification standard.[21] The clinical characteristics of the patients included age, gender, monoclonal protein type, hemoglobin (HGB), platelet (PLT), lactate dehydrogenase (LDH), serum calcium (Ca), albumin (ALB), β2-microglobulin (β2-MG), estimated glomerular filtration rate (eGFR), and ISS stage. The proportion of clonal bone marrow plasma cells (BMPCs) was determined by examining cell morphology and by MFC, and the presence of extramedullary disease was determined by pathological biopsy and immunohistochemical. Immunotyping was performed using MFC, and cytogenetic abnormalities were detected using fluorescence in situ hybridization (FISH). The final follow-up date for all patients was September 30, 2021. The primary endpoints of our study were PFS and OS. PFS was defined as the time from diagnosis to disease progression or death. OS was defined as the time from the date of diagnosis to death from any cause or the last follow-up. The demographics and baseline characteristics of patients with MM are shown in Table 1.

2.2. Multiparameter flow cytometry

Eight-color flow cytometry (Canto II), as produced by BD (Franklin Lakes, NJ, USA) was used for detection with antibodies CD10, CD19, CD20, CD22, CD28, CD45, CD56, CD38, CD138, CD117, kappa, and lambda. Bone marrow fluid (3 mL) was collected from patients with MM and placed in heparin anticoagulant tubes; 100 μL of this sample was then added to a flow cytometry tube containing 20 μL murine anti-human monoclonal antibody and incubated at room temperature in the dark for 20 minute. This solution was then mixed with ammonium chloride for hemolysis and allowed to stand for 15 minute before centrifugation. 10 minute at 1500 r/minute. The supernatant was discarded, and the cells were washed 3 times with phosphate buffered saline. After the above steps were completed, 8-color flow cytometry was performed for detection. Bone marrow cells were classified as CD45/SSC, PCs were delineated by CD38/CD138, and cell surface antigen expression was determined. At diagnosis, ≥20% of PCs were considered positive for antigen expression.

2.3. Fluorescence in situ hybridization

Bone marrow samples of all newly diagnosed MM patients were tested for FISH. The StatSpin ThermoBrite in situ hybridization instrument S500-24 (Abbott Laboratories, Chicago, IL, USA) was used for detection, and the BX53 fluorescence microscope (Olympus, Tokyo, Japan) was used for image reading. Guangzhou Anbiping Pharmaceutical Technology Co. Ltd. (China) probes detected by FISH corresponded to sites 1q21, 17p13 (TP53), 13q14 (RB1, D13S319), 14q32/11q13 (IGH/CCND1), 4p16/14q32 (IGH/FGFR3), and 14q32/16q23 (IGH/MAF). Based on the Mayo Stratification of Myeloma and Risk-Adapted Therapy consensus guidelines,[22] we defined high-risk cytogenetic abnormalities (HRCAs) as gain 1q21, del 17p, t (4;14), and t (14;16), as determined by FISH. Patients with at least two HRCAs were defined as high-risk, and the remaining patients were defined as non-high-risk.

2.4. Chemotherapy regimens and therapeutic effect

All 87 MM patients were hospitalized for treatment. The main chemotherapy regimen was the bortezomib-based combination regimen, with a few patients on the melphalan + prednisone + thalidomide and thalidomide + cyclophosphamide + dexamethasone regimens. When patients reached four to eight cycles of chemotherapy, they were transferred to maintenance therapy. Assessment was performed after four cycles of chemotherapy. According to the IMWG criteria,[23] treatment response was classified into complete response (CR), very good partial response (VGPR), partial response (PR), stable disease (SD) and progressive disease (PD). The objective response rate (ORR) was the sum of CR, VGPR, and PR.

Table 1

Characteristics of 87 newly diagnosed MM patients.

Parameters	Median value (range) or number
Age (yrs)	62 (35–88)
Gender (male)	46 (52.9%)
Monoclonal protein	
IgG	38 (43.7%)
IgA	20 (23.0%)
IgD	3 (3.5%)
Light chain only	25 (28.7%)
Non-secreting type	1 (1.1%)
ISS stage	
I	16 (18.4%)
II	24 (27.6%)
III	47 (54.0%)
HGB (g/L)	87 (39–180)
PLT (10^9/L)	165 (54–361)
LDH (U/L)	208 (92–963)
Serum Ca (mmol/L)	2.45 (1.83–3.50)
ALB (g/L)	33.5 (16.2–51)
β2-MG (mg/L)	5.67 (1.3–30.87)
eGFR (mL/min/1.73 m^2)	49.6 (3.4–163.3)
Clonal BMPCs by morphology (%)	27.5 (0–97.5)
Clonal BMPCs by MFC (%)	16.7 (0.8–81.1)
Presence of extramedullary disease	17 (19.5%)
CD56 expression	54 (62.1%)
FISH	
gain 1q21	34 (39.1%)
del 13q	27 (31.0%)
del 17p	20 (23.0%)
t (4;14)	17 (19.5%)
t (11;14)	5 (5.7%)
t (14;16)	6 (6.9%)

ALB = albumin, β2-MG = β2-microglobulin, BMPCs = bone marrow plasma cells, Ca = calcium, eGFR = estimated glomerular filtration rate, FISH = fluorescence in situ hybridization, HGB = hemoglobin, ISS = International Staging System, LDH = lactate dehydrogenase, MFC = multiparameter flow cytometry, MM = multiple myeloma, PLT = platelet.

2.5. Statistical analyses

Software SPSS 26.0 (IBM) was used for statistical analyses. The counting data were described by frequency and percentage. Comparison between groups was performed by χ^2 test or Fisher exact tests. The Kaplan–Meier method was used to draw the survival curve, and Cox regression analysis was used to analyze survival. $P < .05$ was considered statistically significant.

3. Results

3.1. CD56 expression and patient characteristics

Samples collected from the 87 patients with newly diagnosed MM enrolled in the present study were analyzed using MFC. Patients were divided into 2 groups based on CD56 expression levels: CD56 + (54 cases) and CD56 – (33 cases). As shown in Table 2, no differences in gender, age, HGB, LDH, serum Ca, ALB, β2-MG, or eGFR were observed between the two groups ($P > .05$). The CD56 – group was significantly associated with PLT ($P = .018$). Patients lacking CD56 expression also tended to have an increased incidence of extramedullary disease at diagnosis compared to patients presenting CD56 expression; however, this difference was not significant (29.3% vs 14.8%, $P = .155$). In terms of pathological features, the infiltration rate of malignant bone marrow cells of the CD56 – patients was significantly higher than that of the CD56 + patients (33.3% vs 14.8%, $P = .043$). In addition, according to MFC results, the lack of CD56 expression was significantly correlated with an increased proportion of clonal BMPCs (39.4% vs 18.5%, $P = .032$).

All samples collected from MM patients underwent FISH, and the results are shown in Tables 1 and 2. Of these 87 patients, gain 1q21 was detected in 34, del 13q in 27, del 17p in 20, t (4;14) in 17, t (11;14) in 5, and t (14;16) in 6. There were no significant differences between the CD56 + group and CD56 – group in the above cytogenetic abnormalities ($P > .05$). The proportion of ≥ 2 high-risk cases in the CD56 – group was higher than that in the CD56 + group; however, the difference was not significant ($P > .05$). Possibly due to the small sample size, no significant statistical association was observed between CD56 expression and cytogenetic abnormalities.

3.2. Relationship between CD56 expression and therapeutic effects in MM patients

Our study assessed the response to 4 cycles of bortezomib- or thalidomide-based induction chemotherapy. Four of the 87 MM patients died prematurely or did not reach the end of the 4 cycles and therefore were not included in the efficacy analysis. Of the 83 MM patients assessed for efficacy, 78 received bortezomib-based induction chemotherapy and 5 received thalidomide-based induction chemotherapy. As shown in Table 3, the ORR was 81.1% in the CD56 + group and 60.0% in the CD56 – group, and this difference was statistically significant ($P = .036$). The rate of deep response (VGPR + CR) in the CD56 + group (49%) was higher than that in the CD56 – group (30%), however, this difference was not statistically significant ($P = .091$).

Table 2

CD56 expression and baseline characteristics of patients with MM.

Parameters	CD56 + group (n = 54)	CD56 – group (n = 33)	*P* value
Male	25 (46.3%)	21 (63.6%)	.166
Age at diagnosis ≥ 65 yrs	17 (31.5%)	12 (36.4%)	.639
Monoclonal protein			.154
IgG	25 (46.3%)	13 (39.4%)	
IgA	14 (25.9%)	6 (18.2%)	
IgD	0 (0.0%)	3 (9.1%)	
Light chain only	14 (25.9%)	11 (33.3%)	
Non-secreting type	1 (1.9%)	0 (0.0%)	
ISS stage			.196
I	13 (24.1%)	3 (9.1%)	
II	13 (24.1%)	11 (33.3%)	
III	28 (51.8%)	19 (57.6%)	
HGB < 85 g/L	21 (38.9%)	18 (54.5%)	.154
PLT < 100 × 10^9/L	9 (16.7%)	13 (39.4%)	**.018**
LDH > 250 U/L	16 (29.6%)	14 (42.4%)	.223
Serum Ca > 2.75 mmol/L	15 (27.8%)	14 (42.4%)	.160
ALB < 35 g/L	32 (59.3%)	20 (60.6%)	.901
β2-MG ≥ 5.5 mg/L	28 (51.9%)	19 (57.6%)	.603
eGFR < 40 mL/min/1.73 m^2	19 (35.2%)	10 (30.3%)	.639
BMPCs by morphology > 60%	8 (14.8%)	11 (33.3%)	**.043**
BMPCs by MFC > 30%	10 (18.5%)	13 (39.4%)	**.032**
Extramedullary disease	8 (14.8%)	9 (27.3%)	.155
FISH			
abnormal	36 (66.7%)	27 (81.8%)	.125
gain 1q21	22 (40.7%)	12 (36.4%)	.685
del 13q	17 (31.5%)	10 (30.3%)	.908
del 17p	11 (20.4%)	9 (27.3%)	.458
t (4;14)	9 (16.7%)	8 (24.2%)	.387
t (11;14)	3 (5.6%)	2 (6.1%)	1.000
t (14;16)	2 (3.7%)	4 (12.1%)	.286
≥ 2 high-risk	15 (27.8%)	14 (42.4%)	.160

ALB = albumin, β2-MG = β2-microglobulin, BMPCs = bone marrow plasma cells, Ca = calcium, eGFR = estimated glomerular filtration rate, FISH = fluorescence in situ hybridization, HGB = hemoglobin, ISS = International Staging System, LDH = lactate dehydrogenase, MFC = multiparameter flow cytometry, MM = multiple myeloma, PLT = platelet.

Table 3

Efficacy analysis of chemotherapy after 4 cycles in MM patients.

Efficacy	CD56 + group (n = 53)	CD56 – group (n = 30)	*P* value
ORR	43(81.1%)	18(60.0%)	**.036**
VGPR + CR	26(49.0%)	9(30.0%)	.091
PR	17(32.1%)	9(30.0%)	.845
SD + PD	10(18.9%)	12(40.0%)	**.036**

CR = complete response, MM = multiple myeloma, ORR = overall response rate, PD = progressive disease, PR = partial response, SD = stable disease, VGPR = very good partial response.

3.3. Survival of MM patients

By the end of the follow-up, 38 (43.7%) patients had died, and 58 (66.7%) had relapsed or progressed. The median PFS was 23 months (95% confidence interval [CI], 15.3–30.6) and the median OS was 36 months (95% CI 26.5–45.4). The median PFS was 10 months (95% CI 0.0–21.3) in the CD56 – group and 27 months (95% CI 19.9–34.0) in the CD56 + group (*P* = .007) (Fig. 1A). The median OS of patients in the CD56 – group was 25 months (95% CI 11.4–38.5) versus not reached in the CD56 + group (*P* = .010) (Fig. 1B).

In our study, patients with at least two HRCAs were defined as high-risk and the remaining patients were defined as non-high-risk. When using FISH for risk stratification, the median PFS was 9 months (95% CI 5.0–12.9) in high-risk patients and 32 months (95% CI 25.1–38.8) in non-high-risk patients (*P* < .001) (Fig. 2A). The median OS was 21 months (95% CI 9.1–32.8) in high-risk patients versus not reached in non-high-risk patients (*P* = .008) (Fig. 2B).

Among the high-risk patients, the median PFS was 4 months (95% CI 2.1–5.8) for the CD56 – group and 16 months (95% CI 7.3–24.6) for the CD56 + group (*P* = .012) (Fig. 3A). The median OS of the CD56 + and CD56 – groups was 36 months (95% CI 21.7–50.2) and 15 months (95% CI 6.2–23.7), respectively, with statistically significant differences (*P* = .017) (Fig. 3B). In non-high-risk patients, there were no significant differences in the median PFS (*P* = .293) (Fig. 3C), or median OS (*P* = .361) (Fig. 3D) between the 2 groups.

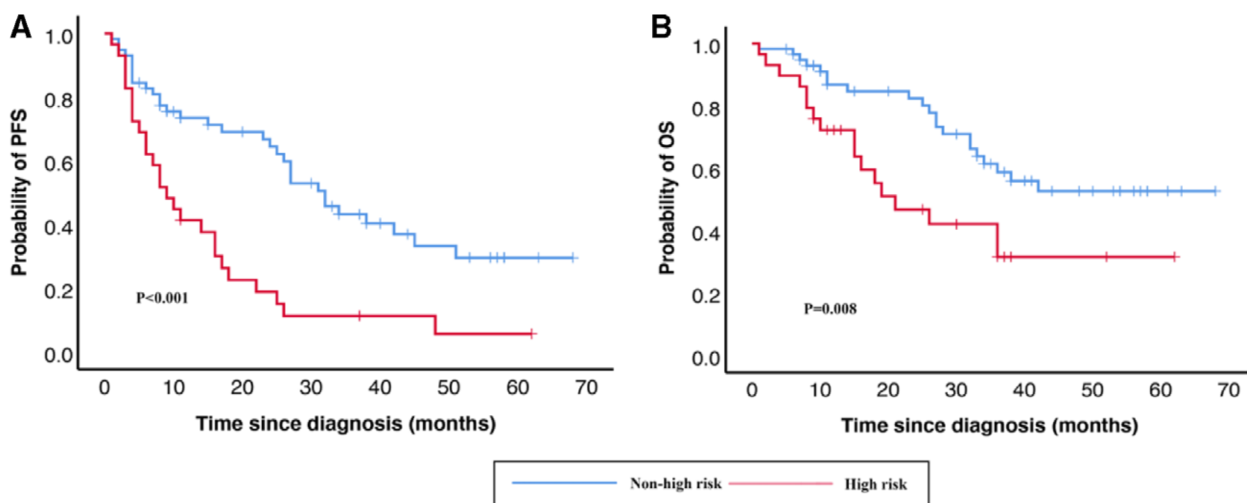

Figure 2. (A) Kaplan–Meier curve comparing PFS between newly diagnosed MM patients based on their molecular cytogenetics classification as high-risk patients versus non-high-risk patients. (B) Kaplan–Meier curve comparing OS between newly diagnosed MM patients based on their molecular cytogenetics classification as high-risk patients versus non-high-risk patients.MM = multiple myeloma, OS = overall survival, PFS = progression-free survival.

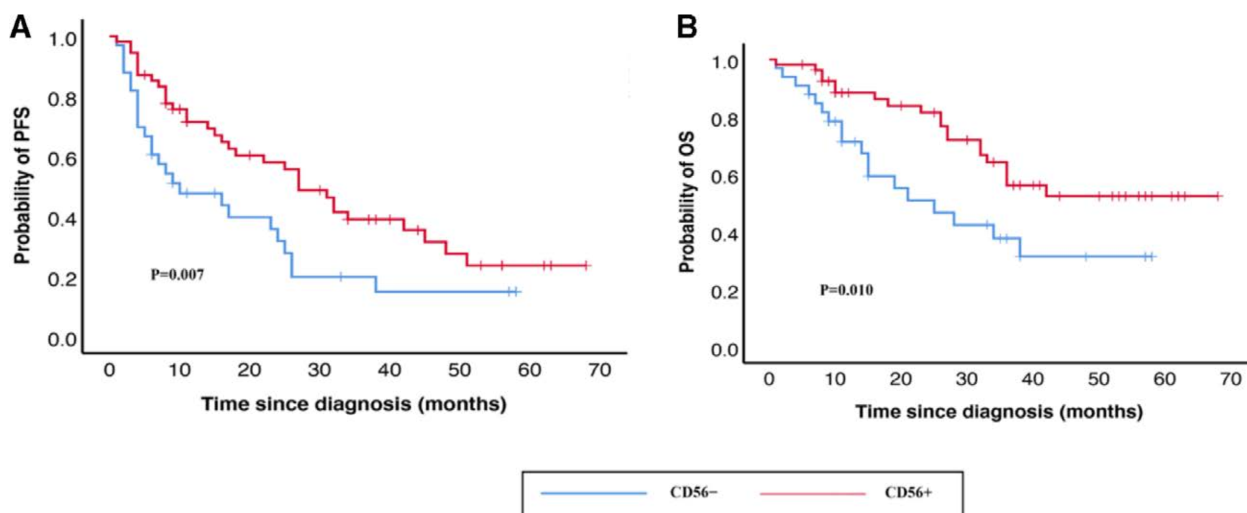

Figure 1. (A) Kaplan–Meier curve comparing PFS between newly diagnosed MM patients based on the expression of CD56. (B) Kaplan–Meier curve comparing OS between newly diagnosed MM patients based on the expression of CD56.MM = multiple myeloma, OS = overall survival, PFS = progression-free survival.

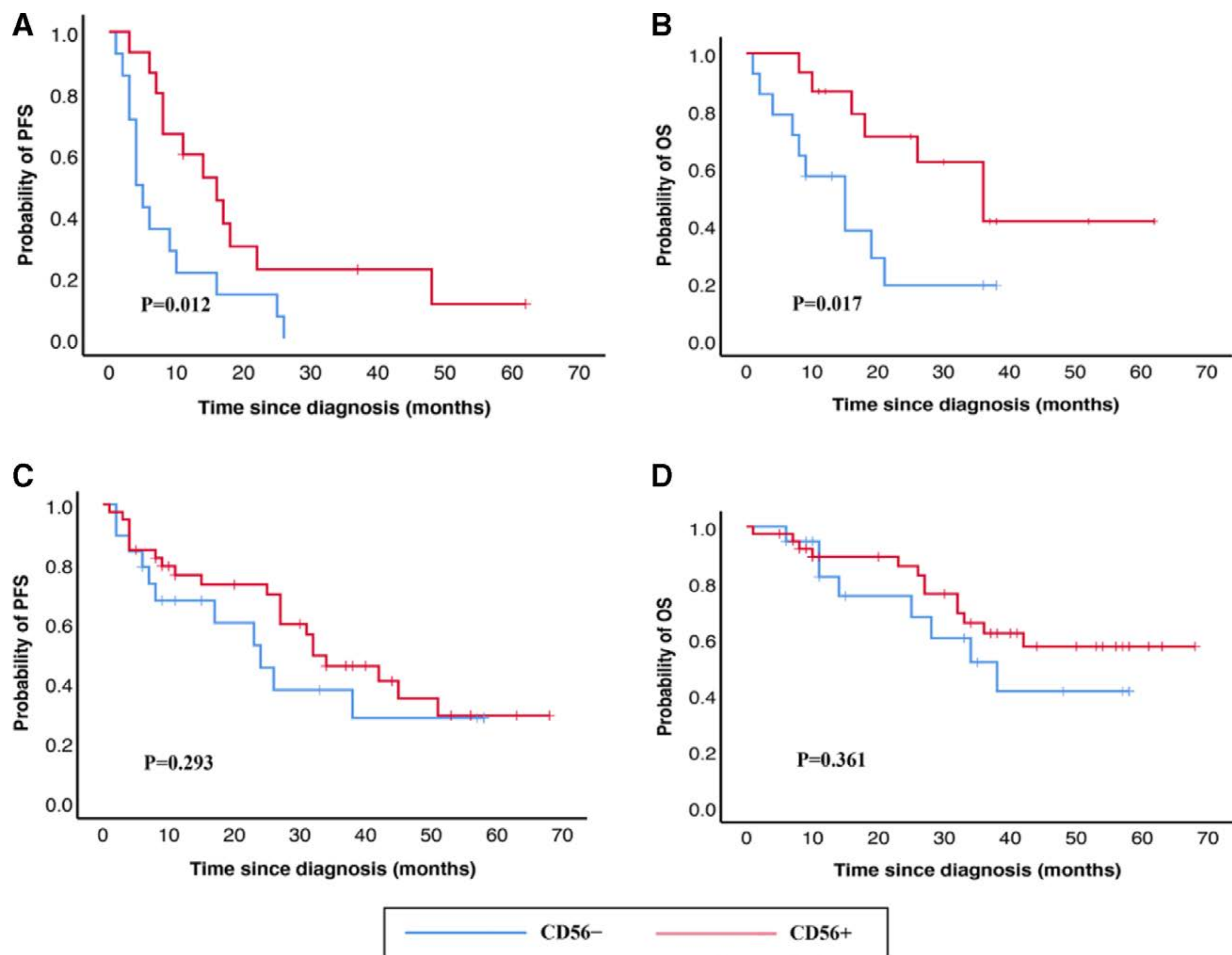

Figure 3. (A)Among the high-risk patients, Kaplan–Meier curve comparing PFS between newly diagnosed MM patients based on the expression of CD56. (B) Among the high-risk patients, Kaplan–Meier curve comparing OS between newly diagnosed MM patients based on the expression of CD56. (C) Among the non-high-risk patients, Kaplan–Meier curve comparing PFS between newly diagnosed MM patients based on the expression of CD56. (D) Among the non-high-risk patients, Kaplan–Meier curve comparing OS between newly diagnosed MM patients based on the expression of CD56.MM = multiple myeloma, OS = overall survival, PFS = progression-free survival.

To further investigate the risk factors affecting the PFS and OS of multiple myeloma patients, we used the Cox proportional risk model for survival analysis. In the univariate analysis, CD56 positivity (HR 0.537, 95% CI 0.318–0.907, $P = .020$), HGB < 85g/L (HR 1.702, 95% CI 1.012–2.861, $P = .045$), LDH > 250U/L (HR 2.347, 95% CI 1.383–3.985, $P = .002$), eGFR < 40 mL/minute/1.73 m^2 (HR 2.160, 95% CI 1.246–3.744, $P = .006$), BMPCs by morphology > 60% (HR 2.397, 95% CI 1.356–4.239, $P = .003$), BMPCs by MFC > 30% (HR 3.436, 95% CI 1.927–6.126, $P < .001$), and FISH ≥ 2 high risk (HR 2.758, 95% CI 1.620–4.694, $P < .001$) were significantly associated with PFS, while gender, age, serum Ca, ALB, β2-MG, and extramedullary disease were not. Further multivariate analysis showed that FISH ≥ 2 high-risk (HR 2.241, 95% CI 1.242–4.044, $P = .007$) was an independent risk factor for PFS (Table 4).

Univariate analysis showed that CD56 positivity (HR 0.465, 95% CI 0.243–0.884, $P = .019$), HGB < 85g/L (HR 2.029, 95% CI 1.066–3.860, $P = .031$), LDH > 250U/L (HR 3.385, 95% CI 1.774–6.460, $P < .001$), eGFR < 40 mL/minute/1.73 m^2 (HR 2.469, 95% CI 1.278–4.773, $P = .007$), BMPCs by morphology > 60% (HR 2.133, 95% CI 1.052–4.322, $P = .036$), BMPCs by MFC > 30% (HR 3.441, 95% CI 1.735–6.632, $P < .001$), and FISH ≥ 2 high-risk (HR 2.230, 95% CI 1.169–4.255, $P = .015$) were all risk factors for OS. In the multivariate analysis, only LDH > 250U/L (HR 2.577, 95% CI 1.230–5.398, $P = .012$) was an independent risk factor for OS (Table 5).

4. Discussion

In recent years, with the development of novel agents such as immunosuppressants and proteasome inhibitors, the prognosis of patients with MM has improved. However, the disease has great heterogeneity and prognosis varies greatly among the different patients. Currently, laboratory indicators and cytogenetics are used by the international community to assess the risk of MM,[21,22] excluding information on abnormal antigen expression. The immunophenotype of tumor cells differs from that of normal cells. MFC has been widely used in the diagnosis of MM, and is also of great significance in monitoring minimal residual disease and in predicting prognosis.[13,24,25] CD56 is expressed in malignant PCs of MM patients.[8] A recent retrospective study found that CD56 expression did not affect the prognosis of MM, but CD56 deficiency was significantly associated with several adverse prognostic factors (LDH, β2-MG, etc).[26] In the era of novel therapeutic agents, the prognostic significance of CD56 in MM patients remains controversial. To further investigate the clinical value of CD56, we explored the correlation between CD56 expression and clinicopathological features, cytogenetic features and patient survival.

In our study, we found that the expression of CD56 was not significantly associated with age, or gender. However, in the study by Ceran et al, CD56 – patients had a lower average age than CD56 + patients (50.2 ± 14.1 and 62 ± 10.3 years,

Table 4

Risk factors for PFS in MM patients.

Characteristic	Univariate analysis HR (95% CI)	*P* value	Multivariate analysis HR (95% CI)	*P* value
CD56				
Negative	1.0(reference)		1.0(reference)	
Positive	0.537 (0.318–0.907)	**.020**	0.744 (0.414–1.339)	.324
Gender				
Female	1.0(reference)			
Male	1.042 (0.621–1.747)	.876		
Age				
<65 yrs	1.0(reference)			
≥65 yrs	1.371 (0.796–2.362)	.255		
HGB				
≥85 g/L	1.0(reference)		1.0(reference)	
<85 g/L	1.702 (1.012–2.861)	**.045**	0.855 (0.435–1.683)	.651
PLT				
≥100 × 10^9/L	1.0(reference)			
<100 × 10^9/L	1.385 (0.784–2.445)	.262		
LDH				
≤250 U/L	1.0(reference)		1.0(reference)	
>250 U/L	2.347 (1.383–3.985)	**.002**	1.803 (0.976–3.333)	.060
Serum Ca				
≤2.75 mmol/L	1.0(reference)			
>2.75 mmol/L	1.480 (0.872–2.512)	.147		
ALB				
≥35 g/L	1.0(reference)			
<35 g/L	1.389 (0.807–2.931)	.236		
β2-MG				
<5.5 mg/L	1.0(reference)			
≥5.5 mg/L	1.389 (0.807–2.931)	.236		
eGFR				
≥40 mL/min/1.73 m^2	1.0(reference)		1.0(reference)	
<40 mL/min/1.73 m^2	2.160 (1.246–3.744)	**.006**	1.440 (0.721–2.875)	.302
BMPCs by morphology				
≤60%	1.0(reference)		1.0(reference)	
>60%	2.397 (1.356–4.239)	**.003**	1.743 (0.900–3.376)	.099
BMPCs by MFC				
≤30%	1.0(reference)		1.0(reference)	
>30%	3.436 (1.927–6.126)	**.000**	1.919 (0.998–3.689)	.051
Extramedullary disease				
No	1.0(reference)		1.0(reference)	
Yes	1.619 (0.847–3.095)	.145	2.241 (1.242–4.044)	**.007**
FISH ≥ 2 high-risk				
No	1.0(reference)			
Yes	2.758 (1.620–4.694)	**.000**		

ALB = albumin, β2-MG = β2-microglobulin, BMPCs = bone marrow plasma cells, Ca = calcium, eGFR = estimated glomerular filtration rate, FISH = fluorescence in situ hybridization, HGB = hemoglobin, LDH = lactate dehydrogenase, MFC = multiparameter flow cytometry, MM = multiple myeloma, PFS = progression-free survival, PLT = platelet.

P = .0032).[27] In addition, they found that CD56 + was more often in stage I to II (65.5%) and CD56 – was more often in stage III (80%) (*P* = .028). In addition, they found that CD56 + was more frequent in stages I and II of MM (65.5%), while CD56 – was more frequent in stage III (80%) (*P* = .028). However, in the present study, no differences were observed between the 2 groups. The different sample sizes between the 2 studies might explain the different results. In laboratory tests, the expression of CD56 was not significantly correlated with monoclonal protein type, HGB, LDH, serum Ca, ALB, β2-MG, eGFR, or extramedullary disease, whereas the absence of CD56 expression was significantly associated with the reduction of PLT. Consistent with our study, previous study has shown that CD56 deficiency in MM is associated with a decreased PLT count.[12] In addition, CD56 – was associated with higher β2-MG, renal dysfunction, monoclonal protein type, presence of urinary light chains, and extramedullary disease.[12] In general, tumor cells are confined to the bone marrow lumen. However, in a few cases, other organs and tissues may be involved. In the present study, 17 patients (19.5%) developed extramedullary disease, with a higher proportion (27.3%) in the CD56 – group than that in the CD56 + group (18.4%). Dahl et al observed that all extramedullary MM cells showed downregulation of CD56,[28] and Kremer et al also found that extramedullary plasmacytoma showed infrequent expression of CD56.[29] Furthermore, Chang et al found that CD56 loss from malignant plasma cells in the cerebrospinal fluid is a hallmark of MM involving the central nervous system.[30] Thus, the low expression of CD56 may enhance the invasion of MM cells and promote extramedullary invasion of tumor cells.

Earlier studies found that the extent of bone marrow infiltration on trephine biopsy was inversely correlated with CD56 expression (*P* = .022).[31] Recently, Ceran et al[27] found that the rate of BMPCs for the CD56 – group was higher than that in the CD56 + group when MFC was used; however, the difference was not statistically significant. In our study, we found that the infiltration rate of malignant marrow cells in the CD56 – group was significantly higher than that in the CD56 + group, either by plasma cells in bone marrow aspiration or by MFC detection. In addition, Koumpis et al[26] found that the lack of CD56 expression was significantly associated with clonal BMPCs infiltration ≥60% (*P* = .009). And mature plasma cells were found

Table 5

Risk factors for OS in MM patients.

Characteristic	*Univariate analysis* HR (95% CI)	*P* value	*Multivariate analysis* HR (95% CI)	*P* value
CD56				
Negative	1.0(reference)		1.0(reference)	
Positive	0.465(0.243–0.884)	**.019**	0.567(0.283–1.138)	.110
Gender				
Female	1.0(reference)			
Male	0.923(0.487–1.747)	.805		
Age				
<65 yrs	1.0(reference)			
≥65 yrs	1.835(0.962–3.499)	.065		
HGB				
≥85 g/L	1.0(reference)		1.0(reference)	
<85 g/L	2.029(1.066–3.860)	**.031**	1.018(0.446–2.323)	.967
PLT				
≥100 × 10^9/L	1.0(reference)			
<100 × 10^9/L	1.840(0.951–3.559)	.070		
LDH				
≤250 U/L	1.0(reference)		1.0(reference)	
>250 U/L	3.385(1.774–6.460)	**.000**	2.577(1.230–5.398)	**.012**
Serum Ca				
≤2.75 mmol/L	1.0(reference)			
>2.75 mmol/L	1.400(0.735–2.666)	.306		
ALB				
≥35 g/L	1.0(reference)			
<35 g/L	1.258(0.643–2.459)	.503		
β2-MG				
<5.5 mg/L	1.0(reference)	.206	1.0(reference)	
≥5.5 mg/L	1.517(0.795–2.895)		1.432(0.631–3.252)	.390
eGFR				
≥40 mL/min/1.73 m^2	1.0(reference)		1.0(reference)	
<40 mL/min/1.73 m^2	2.469(1.278–4.773)	**.007**	1.337(0.603–2.960)	.475
BMPCs by morphology				
≤60%	1.0(reference)		1.0(reference)	
>60%	2.133(1.052–4.322)	**.036**	1.794(0.862–3.735)	.118
BMPCs by MFC				
≤30%	1.0(reference)		1.0(reference)	
>30%	3.441(1.735–6.632)	**.000**	1.694(0.837–3.431)	.143
Extramedullary disease				
No	1.0(reference)			
Yes	1.364(0.624–2.983)	.437		
FISH ≥ 2 high risk				
No	1.0(reference)			
Yes	2.230(1.169–4.255)	**.015**		

ALB = albumin, β2-MG = β2-microglobulin, BMPCs = bone marrow plasma cells, Ca = calcium, eGFR = estimated glomerular filtration rate, FISH = fluorescence in situ hybridization, HGB = hemoglobin, LDH = lactate dehydrogenase, MFC = multiparameter flow cytometry, MM = multiple myeloma, OS = overall survival, PLT = platelet.

in 74% (50/68) of patients with CD56 + and 53% (21/40) of patients with CD56 – in bone marrow biopsies (P = .044). It was further verified that the lack of CD56 expression might be related to the high aggressiveness of MM.

In the present study, after 4 cycles of bortezomib or lenalidomide induction, we found that newly diagnosed MM patients with CD56 deficiency had a poorer response to chemotherapy. More importantly, we found that the median PFS and OS were significantly shorter in patients with CD56 – than in patients with CD56+. These results therefore confirmed that CD56 – MM patients have poor prognosis and that CD56 – is a marker of MM disease progression. Univariate analysis showed that CD56 – was a risk factor for PFS and OS. However, further multivariate analysis showed that CD56 – was not an independent risk factor for PFS and OS.

An previous study showed that MM patients with CD56 negative had shorter OS than patients with CD56 positive,[12] which was later confirmed by Pan et al.[17] Multiple studies have demonstrated that the absence of CD56 expression is a poor prognostic factor, even in the age of new therapeutic agents.[14,18,32–34] Okura et al[18] found that the median OS of CD56 – MM was 24 months, and that of CD56 + MM was 60 months (P = .005). Moreover, in a recent study with 332 MM patients,[34] median OS (58.4 months vs 43.1 months, P = .024) and median PFS (28.7 months vs 24.1 months, P = .013) were significantly higher in CD56 + patients than in CD56 – patients. Takashi et al demonstrated that positive CD56 expression was associated with a better response to bortezomib treatment because the enhanced expression of NCAM triggered endoplasmic reticulum stress and enhanced bortezomib-induced apoptosis of MM cells.[35] Furthermore, Baughn et al found that bortezomib-resistant cells were associated with CD56 deletion,[36] further confirming that CD56 – patients were insensitive to proteasome inhibitor therapy.

However, we obtained different conclusions. In a prospective study by Kraj et al, there was no significant difference in the BMPCs infiltration rate between CD56 – MM patients and CD56 + MM patients, and there was no significant difference in treatment response or survival between the two groups.[15] These differences may be attributed to regional factors. Unlike

our study, the aforementioned study was conducted in Europe. Another report,[16] also from Europe, found that CD56 negativity is not a marker of bad outcome in patients with MM receiving high doses of chemotherapy. Skerget et al observed shorter PFS in CD56 – patients but did not analyze the effect of CD56 expression on OS.[14] Consistent with our hypothesis, a recently published meta-analysis[19] found that CD56 as a prognostic factor for OS was only observed in Asian patients, while the prognostic value of non-Asian patients was not reflected, at least temporarily. Additionally, different detection techniques may affect the experimental results to a certain extent. This suggests that technical aspect and the study area can influence the relationship between CD56 and MM prognosis.

Patients with MM often have abnormal karyotypes. Pozdnyakova et al suggested that CD56 expression is associated with distinct genetic pathways and discovered that CD56 – cases had a higher number of cytogenetic abnormalities (73%) than CD56 + cases (64%).[37] Narita et al observed that all t (14;16)-positive cases were CD56–,[38] suggesting a significant correlation between CD56 negativity and high-risk cytogenetics. Regarding the relationship between cytogenetics and CD56 expression, in our study, 27 (81.8%) CD56 – patients had cytogenetic abnormalities and 14 (42.4%) had ≥ 2 HRCAs. Possibly due to the small sample size, no significant association was observed between CD56 expression and cytogenetic abnormalities. According to a previous report, at least 2 high-risk cytogenetic abnormalities demonstrate poor prognosis in newly diagnosed MM patients.[39] We further classified ≥ 2 high-risk cytogenetic patients into the high-risk group (median OS of 21 months) and the remaining patients into the non-high-risk group (median OS not reached) ($P = .008$). The median PFS was significantly lower in the high-risk group than that in the non-high-risk group (9 vs 32 months, $P < .001$). After the risk subgroup analysis, the median PFS and OS of patients in the CD56 – high-risk group were significantly lower than those in the CD56 + high-risk group. Among the non-high-risk group, the PFS and OS tended to be longer in CD56 + patients, but the difference was not statistically significant. This suggests that among newly diagnosed MM patients with ≥ 2 high-risk cytogenetics, the existence of the CD56 negativity can further identify MM patients with poor PFS and OS. It is worth noting that our sample size was relatively small and larger studies are required to confirm this conclusion.

The 87 patients enrolled in the present study received only chemotherapy; therefore, we were unable to evaluate the effect of autologous hematopoietic stem cell transplantation (aHSCT) on the prognosis of patients. It has been reported that there is no difference in CD56 expression with respect to PFS and OS in patients receiving aHSCT.[14] A recent study of aHSCT mobilization in 94 patients with MM conducted to compare the success rate of mobilization in the CD56 expression group with that in the non-expression group showed that CD56 absence can be a predictive factor of mobilization failure at diagnosis.[40] Studies on whether aHSCT can overcome the negative effects of CD56 absence are insufficient, and a large number of related studies should be conducted to further evaluate the prognosis and treatment of MM.

In this work, we further investigate the clinical characteristics, cytogenetics, and prognosis between CD56 – and CD56 + MM patients. Unlike other studies, our study compared the response of CD56 – and CD56 + MM patients to chemotherapy. We found that newly diagnosed MM patients with CD56 deficiency had a poorer response to chemotherapy. In addition, we divided the patients into high-risk group and non-high-risk group based on FISH tests. This study was the first to find that patients in the high-risk group with CD56 – had significantly lower median PFS and OS than those in the high-risk group with CD56+. Therefore, the existence of the CD56 negativity can further identify MM patients with poor PFS and OS.

The study had several limitations. First, this was a retrospective comparison with a small sample size, and there were unobserved confounders. Second, we failed to evaluate the association between CD56 expression and aHSCT. Third, we did not continuously detect changes in CD56 expression during chemotherapy treatment. Pan et al observed CD56 loss in malignant plasma cells in 4 patients with disease progression.[17] Further analysis of the change in CD56 expression on the surface of myeloma cells before and after treatment and the relationship between the prognosis of the disease may further clarify the clinical significance of CD56 expression in MM patients.

5. Conclusion

In conclusion, CD56 – MM patients were more likely to have myeloid plasma cell infiltration and high-risk cytogenetic abnormalities and had a worse prognosis than CD56 + patients. In addition, among the patients with ≥2 high-risk cytogenetics, the existence of the CD56 negativity can further identify MM patients with poor PFS and OS. Therefore, for CD56 – MM patients, more active and effective therapeutic measures should be considered to improve patient outcomes.

Authors' contributions

Liping Li and Weizhang Shen conceived the study design. Yan Zhao, Lifang Jin, and Meng Zhao acquired the data for the study. Liping Li, An Shang analyzed and interpreted the data. Liping Li drafted the manuscript. Weizhang Shen and Xiaofeng Li revised the manuscript critically. The authors read and approved the final manuscript.

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