RESEARCH ARTICLE

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Evaluation of CD56 and CD117 Double-Positivity as a Predictor of Poor Prognosis in Multiple Myeloma Patients: A Retrospective Analysis

Multipl Miyelom Hastalarında CD56 ve CD117 İkili Pozitifliğinin Kötü Prognoz Belirteci Olarak Değerlendirilmesi: Retrospektif Bir Analiz

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Abstract

Objective: Despite advancements in treatment, multiple myeloma (MM) remains a challenging hematologic malignancy. It is crucial to stratify risk and perform prognostic assessment with various markers, including the expression of cluster of differentiation 56 (CD56) and cluster of differentiation 117 (CD117). However, the relationship of these markers with MM-related survival remains unclear. In this context, the objective of this study was to investigate the prognostic implications of CD56 and CD117 expression and associated clinical features in MM patients.

Materials and Methods: The population of this retrospective single-center study consisted of adult MM patients whose CD56 and CD117 expression levels were analyzed. Patients were divided into four groups according to their immunophenotypes: $CD56^+CD117^-$, $CD56^-CD117^+$, $CD56^+CD117^+$, and $CD56^-CD117^-$. These groups were compared in terms of demographic and clinical characteristics, response to treatment, and survival outcomes.

Results: Of the 168 MM patients included in the study, CD56 positivity, CD117 positivity, CD56 and CD117 double positivity, and CD56 and CD117 double negativity were observed in 57.1%, 38.1%, 21.4%, and 26.2%, respectively. Patients with double positivity had significantly higher cytogenetic risk and significantly lower overall response rate (ORR) compared to other patients (p<0.001 for both). ORR and overall survival (OS) were significantly lower in CD56-positive patients than in CD56-negative patients (p=0.017 and p=0.004, respectively). Mortality rates were significantly higher in CD56-positive and CD117-positive patients than in double-negative patients (p<0.001 and p=0.002, respectively). Double-negative patients had significantly lower ORR and OS and higher mortality than others (p=0.001, p=0.002, and p<0.001, respectively). High cytogenetic risk was found to be an independent predictor of shorter OS (p>0.001).

Öz

Amaç: Tedavideki gelişmelere rağmen, multipl miyelom (MM) zorlu bir hematolojik malignitedir. CD56 ve CD117 ekspresyonu dahil olmak üzere çeşitli belirteçlerle risk sınıflandırması ve prognostik değerlendirme yapmak önemlidir. Ancak bu belirteçlerin MM ile ilişkili sağkalımla ilişkisi halen belirsizdir. Bu bağlamda, çalışmanın amacı MM hastalarında CD56 ve CD117 ekspresyonunun prognostik etkileri ve ilişkili klinik özelliklerin araştırılmasıdır.

Gereç ve Yöntemler: Bu retrospektif tek merkezli çalışmanın popülasyonunu CD56 ve CD117 ekspresyonları analiz edilen erişkin MM hastaları oluşturdu. Hastalar immünofenotipilerine göre dört gruba ayrıldı: $CD56^+CD117^-$, $CD56^-CD117^+$, $CD56^+CD117^+$ ve $CD56^-CD117^-$. Bu gruplar demografik ve klinik özellikler, tedaviye yanıt ve sağkalım sonuçları açısından karşılaştırıldı.

Bulgular: Çalışmaya dahil edilen 168 MM hastasında CD56 pozitifliği, CD117 pozitifliği, CD56 ve CD117 ikili pozitifliği ve ikili negatifliği sırasıyla %57,1, %38,1, %21,4 ve %26,2 saptandı. İkili pozitif hastalarda diğer hastalara göre anlamlı olarak daha yüksek sitogenetik risk ve anlamlı olarak daha düşük genel yanıt oranı (GYO) görüldü (her iki durum için p<0,001). CD56 pozitif hastalarda CD56 negatif hastalara göre GYO ve genel sağkalım (GS) anlamlı olarak daha düşüktü (sırasıyla p=0,017 ve p=0,004). CD56 pozitif ve CD117 pozitif hastalarda ikili negatif hastalara göre mortalite oranları anlamlı olarak daha yüksekti (sırasıyla p<0,001 ve p=0,002). İkili negatif hastalarda diğerlerine göre anlamlı olarak daha düşük GYO ve GS ve daha yüksek mortalite görüldü (sırasıyla p=0,001, p=0,002 ve p<0,001). Yüksek sitogenetik risk kısa GS'nin bağımsız bir öngördürücüsü olarak bulundu (p>0,001).

Sonuç: Çalışmanın bulguları CD56 ve CD117 ikili pozitif MM hastalarının daha kötü prognoz, daha düşük GYO, daha kısa GS ve daha yüksek mortaliteye sahip olduğunu ortaya koydu.

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Abstract

Conclusion: This study's findings revealed that MM patients with CD56 and CD117 double positivity had poorer prognosis, lower ORR, shorter OS, and higher mortality.

Keywords: Multiple myeloma, Cytogenetic abnormalities, CD56, CD117, Survival

Öz

Anahtar Sözcükler: Multipl miyelom, Sitogenetik anormallikler, CD56, CD117, Sağkalım

Introduction

Multiple myeloma (MM) is a common hematologic malignancy characterized by a spectrum of clinical presentations, including anemia, hypercalcemia, renal insufficiency, and osteolytic destruction [1,2]. Despite significant advancements in immunoregulatory drugs and proteasome inhibitors over the years, MM remains an incurable disease, often marked by a short overall survival (OS) [3]. Given the complex pathophysiological interplay affecting MM progression, performing risk stratification and prognostic assessment with various biochemical and cytogenetic markers and antigenic indicators of malignant plasma cells remains pivotal [1].

The diagnosis, follow-up, and prognosis of MM are primarily based on unique abnormal antigens expressed on MM cells, such as cluster of differentiation 56 (CD56) and cluster of differentiation 117 (CD117) [1,2]. CD56 is a glycoprotein and a prominent marker expressed in MM cells [1,4]. CD117 is a tyrosine kinase receptor expressed primarily in hematopoietic progenitor cells [2,5]. The absence of CD56 and CD117 expression in healthy plasma cells indicates that these molecules are tumor-associated markers for MM [6].

The correlation between decreased expression of CD56 and increased likelihood of malignancy in MM is useful in predicting diagnosis and prognosis [1,7]. Several studies have shown that CD56 negativity and CD56 and CD117 double negativity are adverse prognostic indicators for MM patients [1,2,8]. However, there are also studies reporting contrary results [2,3,4]. The discrepancies between studies are often attributed to limitations such as small sample sizes and short follow-up periods [2,3,4].

In this context, this study was carried out to investigate the prognostic implications of CD56 and CD117 expression in MM patients and the clinical features associated with phenotypes based on these expression profiles.

Materials and Methods

Study Design

This research was designed as a single-site retrospective study and received approval from the University of Health Sciences Türkiye Ümraniye Training and Research Hospital's Local Ethics Committee with decision number 275 and protocol code 25/07/23 on August 10, 2023, ensuring adherence to ethical guidelines as stipulated by the Declaration of Helsinki. Given the retrospective nature of this study and the anonymized handling of patient data, the process of obtaining written informed consent from participants was not applicable.

Population and Sample

The study population consisted of all consecutive adult (aged 18 years or older) MM patients with newly diagnosed MM, whose CD56 and CD117 expressions were analyzed using multiparametric flow cytometry in the Department of Hematology between 2016 and 2023. The International Myeloma Working Group diagnosis, risk stratification, and response criteria were used for all MM patients [9]. The prognostic risk stratification of MM patients was performed according to the International Staging System (ISS) [1,10]. While patients with flow cytometry and cytogenetic data on CD56 and CD117 expression at the time of diagnosis were included in the study, patients with missing CD56 or CD117 data or other missing medical information and patients with coexisting autoimmune disorders were excluded from the study.

Immunophenotyping

For immunophenotyping, we expanded our standard antibody panel, which included CD19, CD38, CD45, CD56, and CD138, by integrating monoclonal antibodies against CD20, CD28, and CD117. The immunophenotyping of myeloma cells was accurately determined by a five-color fluorescence panel including FITC, PE, ECD, PC5, and PC7 fluorochromes, analyzed with the Cytomics FC500 system (Beckman Coulter, Indianapolis, IN, USA). Consistent with established protocols, we categorized antigen expression as negative for levels below 20% and positive when 20% or higher [1].

Data Collection

Patients' baseline demographics (age, sex) and clinical characteristics (comorbidities, Eastern Cooperative Oncology Group [ECOG] performance status [PS], ISS stages, MM subtypes, other CD expressions, bony destructions, types of cytogenetic abnormalities) were collected and recorded on a worksheet. Within the scope of laboratory tests carried out at the time of admission, white blood cell and platelet counts, sedimentation rate, and hemoglobin, creatinine, lactate dehydrogenase (LDH), serum albumin, calcium, and β2-microglobulin levels were

measured. Additionally, immunoglobulin-fixed electrophoresis, light-chain protein data, and malignant plasma cell infiltration rate in the bone marrow were noted.

Follow-up Procedure

Upon admission, all patients underwent conventional induction chemotherapy, following a regimen of four to eight cycles that included treatments based on bortezomib and/or lenalidomide, tailored according to individual clinical needs. The effectiveness of these treatments was assessed after the initial four cycles, categorizing patients into five response groups: complete response (CR), very good partial response (VGPR), partial response (PR), stable disease, and progressive disease (PD) [1]. We calculated the overall response rate (ORR) by dividing the sum of patients achieving CR, VGPR, and PR by the total patient count [2]. Furthermore, specific cytogenetic markers such as t(4;14), t(14;16), t(14;20), deletion of 17p, and amplification of 1q were identified as indicators of a higher cytogenetic risk in MM based on the Stratification for Myeloma and Risk-Adapted Therapy [11,12,13]. OS was defined as the duration from diagnosis until death or last follow-up [3].

Study Groups

Patients were divided into four groups according to their immunophenotypes: $CD56^{+}CD117^{-}$, $CD56^{-}CD117^{+}$, $CD56^{+}CD117^{+}$, and $CD56^{-}CD117^{-}$ [1]. These groups were compared in terms of demographic and clinical characteristics, response to treatment, and survival outcomes.

Statistical Analysis

This study aimed to elucidate the prognostic significance of CD56 and CD117 expressions in MM patients by analyzing their impacts on treatment responses and survival outcomes. We meticulously compiled our dataset, categorizing it into continuous variables, which we then evaluated as mean ± standard deviation if they adhered to normal distribution and as median and minimum-maximum values if they did not. Categorical variables were expressed as counts and percentages. To validate the normalcy of our continuous variables, we employed the Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests.

For the assessment of categorical variables across different groups, we utilized Pearson's chi-square test when the expected cell counts exceeded five, Fisher's exact test for cell counts of less than five, and the Fisher-Freeman-Halton test for tables that exceeded the 2x2 format but had cell counts expected to be less than five. When our analysis involved comparing more than two independent groups, we used one-way analysis of variance for normally distributed numerical variables and the Kruskal-Wallis H test for those that deviated from normal distribution. The assessment of differences between groups in parametric and non-parametric settings was facilitated through Games-Howell or Tukey tests and Dwass-Steel-Critchlow-Fligner tests, respectively.

To pinpoint factors capable of significantly influencing OS, we conducted both univariate and multivariate linear regression analyses. Investigated factors included age, sex, ECOG PS, ISS stage, LDH, albumin, calcium, β2-microglobulin, presence of cytogenetic abnormalities, cytogenetic risk, and CD expression profiles. For categorical variables such as sex, we employed dummy coding with male sex serving as the reference category. Multicollinearity was assessed using variance inflation factor analysis, with all values remaining below 10. Each factor's influence on OS was represented by beta coefficients and 95% confidence intervals, with p values denoting statistical significance.

Kaplan-Meier survival analysis was performed to evaluate OS differences between CD56 and CD117 expression groups. The survival curves were compared using the log-rank test.

For statistical computations and analyses, we utilized Jamovi 2.3.28 and JASP 0.18.3 software packages, accepting $p \leq 0.05$ as the threshold denoting statistical significance. This comprehensive approach ensured a robust examination of the data, aiming to provide conclusive insights into the prognostic value of CD56 and CD117 expressions in MM.

Results

The mean age of the 168 MM patients included in the analysis, 48.8% of whom were male while 51.2% were female, was 67.05±9.50 years. CD56 positivity, CD117 positivity, CD56 and CD117 double positivity, and CD56 and CD117 double negativity were observed in 96 (57.1%), 64 (38.1%), 36 (21.4%), and 44 (26.2%) patients, respectively. Sixty (35.7%) and 28 patients (16.7%) had only CD56- or CD117-positive immunophenotypes, respectively.

Demographic characteristics showed no significant disparities among the different groups ($p>0.05$) (Table 1). However, notable distinctions were observed in the incidence of chronic obstructive pulmonary disease and coronary artery disease/heart failure across the groups with statistical significance ($p=0.001$ and $p<0.001$, respectively). Comparisons of ECOG PS and ISS stages across these cohorts revealed no significant variance ($p=0.673$ and $p=0.167$, respectively) (Table 1).

There were significant differences between the groups in terms of LDH and albumin levels ($p=0.010$ and $p=0.018$, respectively). Pairwise comparisons revealed that the patients in the $CD117^{+}$ group had lower LDH values than those in the $CD56^{+}$ group or the $CD56^{-}CD117^{-}$ group ($p=0.006$ and $p=0.045$, respectively).

Table 1. Demographic and baseline clinical characteristics of the study groups.

	CD56$^+$ (n=60)	CD117$^+$ (n=28)	CD56$^+$/CD117$^+$ (n=36)	CD56$^-$/CD117$^-$ (n=44)	p
Age (years)†	66.1±10.1	66.7±10.7	68.7±8.7	67.2±8.5	0.618***
Age groups‡					
<60 years	13 (21.7)	6 (21.4)	5 (13.9)	9 (20.5)	0.805*
≥60 years	47 (78.3)	22 (78.6)	31 (86.1)	35 (79.5)	
Sex†					
Female	28 (46.7)	14 (50.0)	15 (41.7)	25 (56.8)	0.574*
Male	32 (53.3)	14 (50.0)	21 (58.3)	19 (43.2)	
Comorbidities†					
Hypertension	30 (50.0)	12 (42.9)	16 (44.4)	15 (34.1)	0.451*
Diabetes mellitus	20 (33.3)	5 (17.9)	12 (33.3)	10 (22.7)	0.335*
COPD	3 (5.0)[a, b]	5 (17.9)[b, c]	8 (22.2)[c]	0 (0.0)[a]	0.001*
Chronic renal failure	11 (18.3)	3 (10.7)	6 (16.7)	7 (15.9)	0.882*
CAD/heart failure	4 (6.7)[a]	8 (28.6)[b]	0 (0.0)[a]	11 (25.0)[b]	**<0.001***
Others	5 (8.3)	3 (10.7)	3 (8.3)	7 (15.9)	0.661*
ECOG PS‡					
0	16 (26.7)	7 (25.0)	7 (19.4)	9 (20.5)	0.673*
1	19 (31.7)	7 (25.0)	15 (41.7)	17 (38.6)	
2	17 (28.3)	8 (28.6)	12 (33.3)	10 (22.7)	
3	8 (13.3)	6 (21.4)	2 (5.6)	8 (18.2)	
ISS stage‡					
I	12 (20.0)	10 (35.7)	5 (13.9)	17 (38.6)	0.167*
II	19 (31.7)	7 (25.0)	12 (33.3)	9 (20.5)	
III	29 (48.3)	11 (39.3)	19 (52.8)	18 (40.9)	
Subtypes of myeloma‡					
IgG	42 (70.0)	20 (71.4)	23 (63.9)	30 (68.2)	0.925*
IgA	14 (23.3)	6 (21.4)	9 (25.0)	8 (18.2)	
IgM	1 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	
IgD	1 (1.7)	1 (3.6)	3 (8.3)	4 (9.1)	
Light chain only	2 (3.3)	1 (3.6)	1 (2.8)	2 (4.5)	
Light chain‡					
Kappa	39 (65.0)[a]	22 (78.6)[a]	15 (41.7)[b]	18 (40.9)[b]	**0.002***
Lambda	21 (35.0)[a]	6 (21.4)[a]	21 (58.3)[b]	26 (59.1)[b]	
Bone marrow plasma cells (%)§	55.0 [10.0-95.0]	47.5 [15.0-90.0]	67.5 [15.0-90.0]	45.0 [15.0-95.0]	0.473**
Bone lytic destructions†	38 (63.3)[a, b]	16 (57.1)[a, b]	16 (44.4)[b]	33 (75.0)[a]	**0.044***
Other CD expressions†	11 (18.3)	5 (17.9)	8 (22.2)	12 (27.3)	0.692*
Types of other CD phenotypes†					
CD45	5 (45.5)	2 (40.0)	6 (75.0)	5 (41.7)	0.236*
CD19	5 (45.5)	1 (20.0)	0 (0.0)	5 (41.7)	
CD38	1 (9.1)	2 (40.0)	1 (12.5)	2 (16.7)	

†: Mean ± standard deviation; ‡: n (%); §: median [minimum-maximum]; [a, b]: different superscripts indicate statistical differences between groups in each row. There is no statistical difference between groups with the same superscripts; *: Pearson chi-square or Fisher-Freeman-Halton test; **: Kruskal-Wallis H test; ***: One-way analysis of variance. COPD: Chronic obstructive pulmonary disease; CAD: coronary artery disease; ECOG PS: Eastern Cooperative Oncology Group performance status; ISS: International Staging System; Ig: immunoglobulin.

Table 2. Laboratory investigations in groups based on different CD expressions.

	$CD56^+$ (n=60)	$CD117^+$ (n=28)	$CD56^+CD117^+$ (n=36)	$CD56^-CD117^-$ (n=44)	p
Hemoglobin (g/dL)†	10.5±2.4	10.8±1.7	9.7±1.8	10.6±2.0	0.073**
White blood cell count ($x10^3$/L)§	7005.0 [2710.0-25260.0]	6165.0 [2060.0-16000.0]	6660.0 [1400.0-18990.0]	6025.0 [990.0-44800.0]	0.269*
Platelet count ($x10^9$/L)§	196.0 [32.0-421.0]	182.5 [42.0-446.0]	189.5 [9.0-610.0]	208.5 [42.0-512.0]	0.743*
Sedimentation rate (mm/h)§	55.5 [2.0-123.0]	47.5 [2.0-120.0]	56.5 [13.0-112.0]	54.5 [9.0-119.0]	0.480*
Creatinine (mg/dL)§	1.3 [0.5-8.3]	1.0 [0.6-5.5]	1.7 [0.4-6.0]	1.3 [0.4-6.0]	0.235*
Lactate dehydrogenase (U/L)§	301.5 [118.0-2830.0]	240.5 [123.0-2714.0]	287.5 [105.0-1120.0]	293.5 [132.0-747.0]	0.010*
Albumin (mg/dL)§	3.3 [2.2-4.7]	3.5 [2.3-4.8]	3.2 [1.6-4.6]	3.8 [2.5-4.6]	0.018*
Calcium (mmol/L)§	9.4 [7.6-15.3]	9.1 [7.2-16.4]	9.1 [7.2-11.8]	9.0 [7.5-13.9]	0.101*
β2-microglobulin (mg/L)§	5.5 [1.7-57.8]	4.7 [1.2-15.0]	5.5 [1.7-34.6]	4.0 [1.7-22.0]	0.194*

†: Mean ± standard deviation; §: median [minimum-maximum]; *: Kruskal-Wallis H test; **: One-way analysis of variance.

Table 3. Frequencies of cytogenetic abnormalities (n=160) detected in 139 patients.

Cytogenetic abnormality	n (%)
Karyotype‡	
Hypodiploidy	20 (12.5)
Hyperdiploidy	17 (10.6)
Primary immunoglobulin heavy-chain gene translocations‡	
t(6;14)	23 (14.4)
t(11;14)	17 (10.6)
t(4;14)	8 (5)
t(14;20)	6 (3.8)
t(14;16)	4 (2.5)
t(8;14)	2 (1.3)
Secondary cytogenetic abnormalities‡	
del 17P	12 (7.5)
5q amplification	11 (6.9)
1q amplification	9 (5.6)
del 1P	8 (5)
del 13 metaphases	5 (3.1)
RB1 loss	16 (10)
Complex karyotype anomaly‡	2 (1.3)

‡: n (%).

In addition, the patients in the $CD56^+CD117^+$ group had significantly lower albumin levels than those in the $CD56^-CD117^-$ group (p=0.007) (Table 2).

A total of 160 different cytogenetic abnormalities were detected in 139 (82.7%) patients (Table 3). The most common cytogenetic abnormality was t(6;14) (14.4%), followed by hypodiploidy (12.5%).

Although the rate of patients with cytogenetic abnormality was significantly higher in the $CD56^+CD117^+$ group than in the $CD56^+$ and $CD56^-CD117^-$ groups (p=0.030), the comparison of the groups in terms of number of cytogenetic abnormalities (<3 vs. ≥3) revealed no significant difference between them (p=0.295). The rate of patients with high cytogenetic risk was also significantly increased in the $CD56^+CD117^+$ group compared to other groups (p<0.001) (Table 4).

There was no significant difference between the groups in terms of medications used within the scope of first-, second-, and third-line treatments (p>0.05) (Table 5). There was a significant difference between the groups in the frequencies of the treatment response (p<0.001). Accordingly, ORR was significantly lower in the $CD56^+CD117^+$ group than in other groups (p=0.001).

The Kaplan-Meier survival analysis revealed significant differences in overall survival patterns among different CD expression groups (Table 6). Accordingly, the rates of patients with PD and mortality were significantly increased in the $CD56^+$ group than in the $CD56^-$ group (p=0.001 and p<0.001, respectively). In parallel, ORR and OS were significantly lower in the $CD56^+$ group than in the $CD56^-$ group (p=0.017 and p=0.004, respectively) (Figure 1a). There were also significant differences between the $CD117^+$ and $CD117^-$ groups in terms of treatment response (p<0.05). Accordingly, ORR was significantly lower in the $CD117^+$ group than in the $CD117^-$ group (p=0.021). On the other hand, there was no significant difference between these groups for OS (p=0.409) (Figure 1b). The mortality rate was significantly lower in the $CD117^+$ group than in the $CD117^-$ group (p=0.002).

The median OS was significantly higher in the $CD117^+$ group than in the $CD56^+CD117^+$ group (42.0 vs. 11.5 months, p=0.002). The OS in the $CD56^+CD117^+$ group was significantly lower than those of other groups, as well (p=0.002) (Figure 2). In parallel, the mortality rate was significantly elevated in the $CD56^+CD117^+$ group compared to other groups (p<0.001) (Table 7).

Table 4. Cytogenetic abnormalities of the patients.

	CD56+ (n=60)	CD117+ (n=28)	CD56+CD117+ (n=36)	CD56-CD117- (n=44)	p*
Cytogenetic abnormalities†	45 (75.0)[a]	24 (85.7)[a, b]	35 (97.2)[b]	35 (79.5)[a]	0.030
Number of cytogenetic abnormalities†					
<3	55 (91.7)	28 (100.0)	33 (91.7)	43 (97.7)	0.295
≥3	5 (8.3)	0 (0.0)	3 (8.3)	1 (2.3)	
Cytogenetic risk†					
Standard risk	41 (68.3)[a]	23 (82.1)[a]	8 (22.2)[b]	31 (70.5)[a]	**<0.001**
High risk	19 (31.7)[a]	5 (17.9)[a]	28 (77.8)[b]	13 (29.5)[a]	
Autologous hematopoietic stem cell transplantation†	24 (40.0)	14 (50.0)	19 (52.8)	24 (54.5)	0.446

†: n (%). [a, b]: Different superscripts indicate statistical differences between groups in each row. There is no statistical difference between groups with the same superscripts. *: Pearson chi-square or Fisher-Freeman-Halton test.

Table 5. Treatment details and outcomes.

	CD56+ (n=60)	CD117+ (n=28)	CD56+CD117+ (n=36)	CD56-CD117- (n=44)	p*
First-line†					0.710
Bortezomib-based	44 (73.3)	20 (71.4)	25 (69.4)	36 (81.8)	
Combination of bortezomib and thalidomide	2 (3.3)	2 (7.1)	4 (11.1)	1 (2.3)	
Combination of bortezomib and lenalidomide	2 (3.3)	0 (0.0)	0 (0.0)	1 (2.3)	
Others	12 (20.0)	6 (21.4)	7 (19.4)	6 (13.6)	
Second-line†					0.425
Bortezomib-based	22 (44.0)	12 (44.4)	9 (42.9)	17 (41.5)	
Combination of bortezomib and lenalidomide	15 (30.0)	8 (29.6)	6 (28.6)	18 (43.9)	
Combination of bortezomib and thalidomide	5 (10.0)	6 (22.2)	1 (4.8)	3 (7.3)	
Lenalidomide-based	7 (14.0)	1 (3.7)	4 (19.0)	3 (7.3)	
Thalidomide-based	0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)	
Others	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Third-line†					**0.049**
Lenalidomide-based	23 (74.2)	6 (40.0)	2 (20.0)	15 (62.5)	
Combination of bortezomib and lenalidomide	4 (12.9)	5 (33.3)	5 (50.0)	6 (25.0)	
Bortezomib-based	2 (6.5)	3 (20.0)	1 (10.0)	3 (12.5)	
Combination of bortezomib and thalidomide	1 (3.2)	0 (0.0)	1 (10.0)	0 (0.0)	
Others	1 (3.2)	1 (6.7)	1 (10.0)	0 (0.0)	

†: n (%); *: Fisher-Freeman-Halton test.

Univariate regression analysis revealed that cytogenetic risk and CD56 and CD117 co-positivity were poor prognostic factors for OS (p<0.001 and p=0.002, respectively). Further analysis of these two factors with multivariate regression analysis revealed cytogenetic risk as the only factor that could significantly predict OS (odds ratio: -20.13, confidence interval: -28.4 to 11.87, p<0.001) (Table 8).

Discussion

Our study's findings indicate that MM patients with CD56 and CD117 double positivity had significantly lower ORR, shorter OS, and higher mortality rates than those who were either CD56- or CD117-positive and those with CD56 and CD117 double negativity. While univariate analysis revealed high cytogenetic risk and double positivity for CD56 and CD117 as poor prognostic factors for OS, multivariate analysis revealed high cytogenetic risk as the only factor that could significantly predict shorter OS in MM patients.

The prevalence of MM patients with different CD56 and CD117 immunophenotypes has been investigated in numerous studies. These studies are consistent in that they found that approximately two-thirds and one-third of MM patients were

Table 6. Treatment outcomes and survival data of patients according to CD56 and CD117 status.

	CD56+		p	CD117+		p
	No (n=72)	Yes (n=96)		No (n=104)	Yes (n=64)	
Treatment response†						
Complete response	15 (20.8)[a]	13 (13.5)[a]		16 (15.4)[a]	12 (18.8)[a]	
Very good partial response	24 (33.3)[a]	24 (25.0)[a]		38 (36.5)[a]	10 (15.6)[b]	
Partial response	6 (8.3)[a]	4 (4.2)[a]	**0.001***	7 (6.7)[a]	3 (4.7)[a]	**0.007***
Stable disease	13 (18.1)[a]	8 (8.3)[a]		15 (14.4)[a]	6 (9.4)[a]	
Progressive disease	14 (19.4)[a]	47 (49.0)[b]		28 (26.9)[a]	33 (51.6)[b]	
Overall response rate (%)†	45 (62.5)	41 (42.7)	**0.017***	61 (58.7)	25 (39.1)	**0.021***
Overall survival (months)§	42.0 [1.0-132.0]	25.0 [1.0-93.0]	**0.004****	34.0 [1.0-93.0]	27.5 [1.0-132.0]	0.236**
Progression†	70 (97.2)	92 (95.8)	0.701*	99 (95.2)	63 (98.4)	0.409*
Outcome†						
Survived	58 (80.6)	49 (51.0)	**<0.001***	76 (73.1)	31 (48.4)	**0.002***
Deceased	14 (19.4)	47 (49.0)		28 (26.9)	33 (51.6)	

†: n (%); §: median [minimum-maximum]; [a, b]: different superscripts indicate statistical differences between groups in each row. There is no statistical difference between groups with the same superscripts; *: Pearson chi-square or Fisher-Freeman-Halton test; **: Kruskal-Wallis H test.

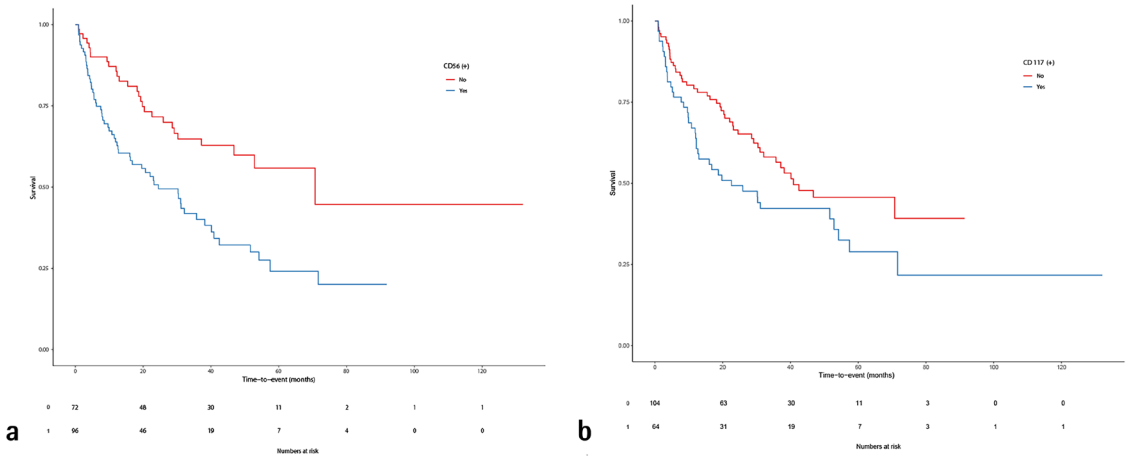

Figure 1. Kaplan-Meier curves for overall survival according to patients' (a) CD56 and (b) CD117 statuses.

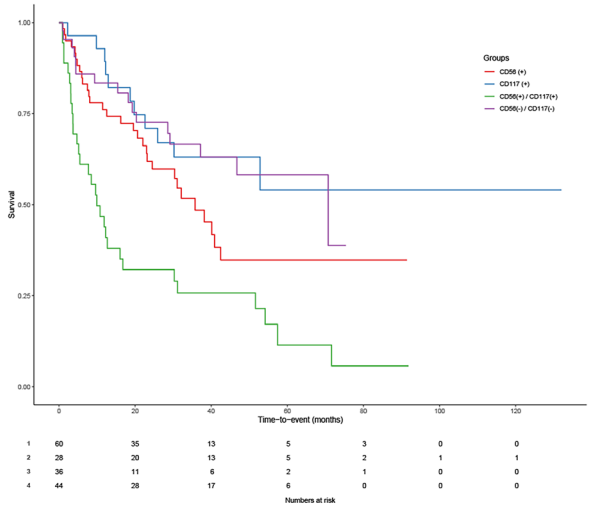

Figure 2. Kaplan-Meier curves for overall survival by groups.

CD56-positive and CD117-positive, respectively [6]. In addition, CD56 and CD117 double negativity and CD56 and CD117 double positivity were detected in almost one-quarter to one-third of patients with MM [1,2,14]. However, there are also studies reporting lower incidences of CD117 positivity [15,16]. The discrepancies between studies in rates of MM patients with different CD56 and CD117 levels may be attributed to methodological variations, suggesting no significant impact on the reliability of outcomes [5].

The impact of CD56 and CD117, together with cytogenetic abnormalities, on the survival of MM patients has been investigated in previous studies. In one such study, Zheng et al. [1] found that CD56 negativity was a poor prognostic marker associated with increased adverse cytogenetic abnormalities and that CD56 and CD117 double negativity was associated

Table 7. Treatment outcomes and survival data of the groups.

	CD56$^+$ (n=60)	CD117$^+$ (n=28)	CD56$^+$CD117$^+$ (n=36)	CD56$^-$CD117$^-$ (n=44)	p
Treatment response†					
Complete response	8 (13.3)^a	7 (25.0)^a	5 (13.9)^a	8 (18.2)^a	**<0.001***
Very good partial response	21 (35.0)^a	7 (25.0)$^{a, b}$	3 (8.3)^b	17 (38.6)^a	
Partial response	4 (6.7)$^{a, b}$	3 (10.7)^b	0 (0.0)^a	3 (6.8)$^{a, b}$	
Stable disease	8 (13.3)^a	6 (21.4)^a	0 (0.0)^b	7 (15.9)^a	
Progressive disease	19 (31.7)^a	5 (17.9)^a	28 (77.8)^b	9 (20.5)^a	
Overall response rate (%)†	33 (55.0)^a	17 (60.7)^a	8 (22.2)^b	28 (63.6)^a	**0.001***
Overall survival (months)§	32.0 [1.0-93.0]	42.0 [2.0-132.0]	11.5 [1.0-93.0]	39.0 [1.0-91.0]	**0.002****
Progression†	57 (95.0)	28 (100.0)	35 (97.2)	42 (95.5)	0.845*
Outcome†					
Survived	41 (68.3)^a	23 (82.1)^a	8 (22.2)^b	35 (79.5)^a	**<0.001***
Deceased	19 (31.7)^a	5 (17.9)^a	28 (77.8)^b	9 (20.5)^a	

†: n (%); §: median [minimum-maximum]; $^{a, b}$: different superscripts indicate statistical differences between groups in each row. There is no statistical difference between groups with the same superscripts; *: Pearson chi-square or Fisher-Freeman-Halton test; **: Kruskal-Wallis H test.

Table 8. Impact of demographic, clinical, and molecular factors on overall survival.

	Univariate linear regression		Multivariate linear regression	
Variables	**Beta coefficient [95% CI]**	**p value**	**Beta coefficient [95% CI]**	**p value**
Age	0.22 [-0.19 to 0.62]	0.295	-	-
Sex: female vs. male	-3.38 [-10.98 to 4.22]	0.384	-	-
ECOG PS: 2-3 vs. 0-1	-2.35 [-10.05 to 5.35]	0.551	-	-
ISS stage: III vs. I-II	-2.20 [-9.84 to 5.44]	0.573	-	-
Lactate dehydrogenase	-0.01 [-0.02 to 0.01]	0.065	-0.01 [-0.02 to 0.01]	0.167
Albumin	3.13 [-2.33 to 8.60]	0.263	-	-
Calcium	-0.51 [-3.13 to 2.11]	0.703	-	-
β2-microglobulin	0.01 [-0.52 to 0.51]	0.988	-	-
Cytogenetic abnormalities: present vs. absent	-7.37 [-17.39 to 2.64]	0.151	3.35 [-6.48 to 13.19]	0.505
Cytogenetic risk: high risk vs. standard risk	-21.43 [-28.53 to -14.32]	<0.001*	-20.13 [-28.4 to -11.87]	<0.001*
CD expressions: CD56$^+$/CD117$^+$ vs. others	-14.59 [-23.61 to -5.58]	0.002*	-4.83 [-14.12 to 4.47]	0.311
Autologous hematopoietic stem cell transplantation: absent vs. present	1.16 [-6.21 to 8.54]	0.756	-	-
First line treatment regimens: non-bortezomib vs. bortezomib based	22.15 [-5.47 to 49.8]	0.115	-	-

*Statistically significant (p<0.05). ECOG PS: Eastern Cooperative Oncology Group performance status; ISS: International Staging System; CI: confidence interval.

with worse clinical outcomes. Similar findings were reported in other studies [17,18,19,20]. It was reported that CD56 positivity was significantly associated with higher ORR and OS compared to those CD56 negativity [14,21]. In a meta-analysis, Zhang et al. [18] concluded that CD56 negativity was associated with poorer OS and progression-free survival (PFS) in MM patients. In sum, discrepancies exist between these studies, possibly resulting from differences in treatment regimens, diagnostic methods, survival analyses, region, and cutoff values for determining CD56 expression-based immunophenotypes [11,12,18,22,23,24,25,26].

Contrary to the studies that found CD117 positivity as an independent poor prognostic factor for PFS in MM patients [2,3,15], some studies speculated that CD117 positivity alone might be a marker for good prognosis. Nevertheless, there are also discrepancies between studies on survival outcomes of MM patients with various CD expression-based immunophenotypes [5,6,16,27]. In comparison, we did not observe a significant impact of CD117 expression alone on patients' survival.

The comparison of survival outcomes of MM patients according to CD56 or CD117 positivity and CD56 and CD117 double positivity and double negativity revealed significantly lower

OS in the $CD56^{-}CD117^{-}$ group than in other groups [1,2,14]. In contrast, Wang et al. [3] found no significant difference in survival between $CD56^{+}CD117^{+}$ and $CD56^{-}CD117^{-}$ groups in their study. In some studies, survival analysis could not be performed due to the small sample size because there were not enough patients in the CD expression-based immunophenotype subgroups [2,8]. The differences observed in the prevalence of high-risk cytogenetic abnormalities might be associated with the biological characteristics of MM, rendering the prognostic impacts of immunophenotype-based indicators controversial [5]. Therefore, further large-scale studies are needed to elucidate the prognostic values of immunophenotype-based indicators.

Various risk factors have been reported in patients with MM in the literature, including adverse cytogenetic abnormalities, LDH, β2-microglobulin, anemia, bone marrow plasma cells, and impaired renal function [1,2,14]. Although the mechanism involved is still unclear, CD56 negativity has been associated with some poor prognostic factors, such as high serum β2-microglobulin level, low platelet count, high anemia incidence, and renal failure [2]. Shi et al. [14] found that higher serum creatinine levels and CD56 negativity were independent risk factors for OS in patients with MM. In comparison, we found that CD56 and CD117 double positivity and high-risk cytogenetic abnormalities were associated with shorter OS, and among the two, high-risk cytogenetic abnormalities were a factor for significantly poorer prognosis.

The relationship between critical cytogenetic abnormalities and survival outcomes in the context of MM is well established. It has been reported in the literature that cytogenetic abnormalities such as t(4;14), t(14;16), t(14;20), 17p deletion, and 1q amplification are associated with poor prognosis. In addition, several authors have considered that the complexity of the bone marrow microenvironment, oncogene overexpression, and genomic instability may also complicate the adverse effects of cytogenetic abnormalities [1,28]. Zheng et al. [1] found that t(4;14) translocations were significantly more common in CD56-negative patients. In contrast, we did not compare MM patients in different CD expression-based immunophenotype subgroups according to different cytogenetic abnormalities. The lack of a standardized classification system for cytogenetic abnormalities in MM patients makes analysis of the effects of cytogenetic abnormalities on prognosis difficult.

Study Limitations

This study's primary limitation was its retrospective single-center design and relatively small sample size. The fact that we could not compare the survival outcomes of CD expression-based immunophenotype subgroups according to cytogenetic abnormalities due to the lack of a standardized classification of cytogenetic abnormalities in MM and the insufficient number of patients with different abnormalities can also be considered a limitation. Another limitation was that we could not perform conventional cytogenetic analysis via G-banding due to its high cost. In addition, our CD expression-based immunophenotyping was based solely on analysis of CD expression at the time patients were first diagnosed with MM. Finally, we could not address the potential impact of different chemotherapeutics and novel agents.

Conclusion

This study's findings revealed that MM patients with CD56 and CD117 double positivity had poorer prognosis, lower ORR, shorter OS, and higher mortality. Therefore, CD56 and CD117 co-positivity can be used as a poor prognostic marker for MM. Future large-scale studies are needed to elucidate the roles of tumor-associated markers on survival outcomes of MM patients.

Ethics

Ethics Committee Approval: This research was designed as a single-site retrospective study and received approval from the University of Health Sciences Türkiye Ümraniye Training and Research Hospital's Local Ethics Committee with decision number 275 and protocol code 25/07/23 on August 10, 2023, ensuring adherence to ethical guidelines as stipulated by the Declaration of Helsinki.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: H.K.; Concept: S.M., H.K.; Design: H.K., I.E.Z.; Data Collection or Processing: S.M., I.E.Z.; Analysis or Interpretation: S.M., H.K.; Literature Search: H.K., I.E.Z.; Writing: S.M., H.K., I.E.Z.

Conflict of Interest: No conflict of interest was declared by the authors.

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