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

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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 through various markers, including the cluster of differentiation 56 (CD56) and thecluster of differentiation 117 (CD117) expression. However, the relationship of these markers with MM-related survival remains unclear. In this context, the objective of this study isto 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 expressionswere 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 positive, CD117 positive,CD56, and CD117 double-positive and double negative were observed in 57.1%, 38.1%, 21.4%, and 26.2%, respectively. Patients with double positivehad significantly higher cytogenetic risk and significantly lower overall response rate (ORR) compared toother patients($p<0.001$ for both cases). ORR and overall survival (OS) were significantly lower in CD56 positivepatients than those CD56 negative ($p=0.017$ and $p=0.004$, respectively). Mortality rates were significantly higher in CD56 positive patients and CD117 positive than in those with double negative($p<0.001$ and $p=0.002$). 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). The high cytogenetic risk was found to bean independent predictor of shorter OS ($p>0.001$).
Conclusion: The study's findings revealed that CD56 and CD117 double- positive MM patients had poorer prognosis, lower ORR, shorter OS, and higher mortality.
Keywords: Multiple myeloma, cytogenetic abnormalities, CD56, CD117, survival

Introduction

Multiple myeloma (MM) is a common hematologic malignancy characterized by a spectrum of clinical presentations, includinganemia, 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[3]. Given the complex pathophysiological interplay affecting MM progression, risk stratification and prognostic assessment through various biochemical, cytogenetic markers, and antigenic indicators of malignant plasma cells remain pivotal [1].
Diagnosis, follow-up, and prognosis of MM are made primarily based on unique, abnormal antigens expressed on MM cells, such as the cluster of differentiation 56 (CD56) and the cluster of differentiation 117 (CD117)[1,2]. CD56 is a glycoprotein anda 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 aretumor-associated markers for MM [6].

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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-negativeand CD56 and CD117 double-negativeare adverse prognostic indicators for MM patients [1,2,8]. However, there are also studies reporting contrary results [2–4]. The discrepancies between the studies are often attributed to limitations such as small sample sizes and short follow-up periods [2–4].

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

Materials and Methods

Study Design

This research was executed as a single-site retrospective study and received approval from the institutional review board, 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 or older) MM patientswith newly diagnosed MM, whose CD56 and CD117 expressions were analyzed using multiparametric flow cytometry at the Department of Hematology between 2016 and 2023.The International Myeloma Working Group (IMWG) 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 expressions at the time of diagnosis were included in the study, patients with missing CD56 or CD117 data and 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 immunophenotypingof myeloma cells was accurately determined by a five-color fluorescence panel including FITC, PE, ECD, PC5, and PC7 fluorochromes, analyzed by the Beckman Coulter Cytomics FC500 system. 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 demographic (age, gender) and clinical characteristics [comorbidities, the Eastern Cooperative Oncology Group (ECOG) performance status, ISS stages, MM subtypes, other CD expressions, bony destructions, the types of cytogenetic abnormalities] were collected and recorded into a worksheet. Within the scope of laboratory tests carried out at the time of admission, white blood cell (WBC) and platelet counts, sedimentation rate, hemoglobin, creatinine, lactate dehydrogenase (LDH), serum albumin, calcium and β2-microglobulin (2-MG) levels were measured. Additionally, immunoglobulin-fixed electrophoresis, light chain protein data, and the malignant plasma cell infiltration rate in the bone marrow were collected.

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 (SD), 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 (mSMART) [11–13]. Overall survival (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 multiple myeloma (MM) patients by analyzing their impacts on treatment responses and survival outcomes. We meticulously compiled our data set, categorizing it into continuous variables, which we then delineated by mean ± standard deviation if they adhered to a normal distribution, and by median along with minimum and 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.

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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 cells with fewer than five, and the Fisher-Freeman-Halton test for tables that exceeded the 2x2 format but had cells expected to contain less than five. When our analysis involved comparing more than two independent groups, we turned to the one-way analysis of variance (ANOVA) 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 overall survival (OS), we conducted both univariate and multivariate linear regression analyses. Investigated factors included age, gender, Eastern Cooperative Oncology Group Performance Status (ECOG-PS), International Staging System (ISS) stage, levels of lactate dehydrogenase, albumin, calcium, and β2-microglobulin, presence of cytogenetic abnormalities, cytogenetic risk, and CD expression profiles. Each factor's influence on OS was represented by beta coefficients and 95% confidence intervals, with p-values denoting statistical significance.

For statistical computations and analyses, we utilized the Jamovi project 2.3.28 and JASP 0.18.3 software packages, embracing a p-value threshold of ≤ 0.05 to denote statistical significance. This comprehensive approach ensured a robust examination of the data, aiming to draw conclusive insights into the prognostic value of CD56 and CD117 expressions in MM.

Results

The mean age of 168 MM patients included in the sample, of whom 48.8% were male and 51.2% were female, was 67.05 ± 9.50 years. CD56 positive, CD117 positive, CD56, and CD117 double-positive, and double-negative 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.

The demographic attributes 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 (COPD) and coronary artery disease (CAD)/heart failure across the groups, with statistical significance ($p=0.001$ and $p<0.001$, respectively). Comparisons of Eastern Cooperative Oncology Group Performance Status (ECOG-PS) and International Staging System (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 LDH and albumin levels($p=0.010$ and $p=0.018$, respectively). Pairwise comparisons revealed that the patients in Group $CD117^+$ had lower LDH values than those in Group $CD56^+$ and Group $CD56^-CD117^-$ ($p=0.006$ and $p=0.045$, respectively). In addition, the patients in Group $CD56^+CD117^+$ had significantly lower albumin levels than those in group$CD56^-CD117^-$($p=0.007$)(**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 byhypodiploidy (12.5%).

Although the rateof patients with cytogenetic abnormality wassignificantly higher in Group $CD56^+CD117^+$than in Groups $CD56^+$ and $CD56^-CD117^-$($p=0.030$), the comparison of the groups inthe number of cytogenetic abnormalities (<3 vs. ≥3) revealed no significant difference between the groups ($p=0.295$). The rateof patients with high cytogenetic risk wasalso significantly increased in group $CD56^+CD117^+$than in 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 group $CD56^+CD117^+$than in other groups ($p=0.001$).

There weresignificant differences between the groups in terms of survival outcomes (Table 6). Accordingly, the rates of patients with progressive disease and mortality were significantly increasedin group $CD56^+$than in group $CD56^-$($p=0.001$ and $p<0.001$, respectively). In parallel, the ORR and OS were significantly lower in $CD56^+$than in group $CD56^-$($p=0.017$ and $p=0.004$, respectively) (**Fig.1a**). There were also significant differences between Group $CD117^+$ and Group $CD117^-$in treatment response ($p<0.05$). Accordingly, ORR was significantly lower in Group $CD117^+$than in group $CD117^-$ ($p=0.021$). On the other hand, there was no significant difference between the said groups in OS ($p=0.409$) (**Fig. 1b**). The mortality rate was significantly lower in group $CD117^+$than in group $CD117^-$($p=0.002$).

The median OS was significantly raised in group $CD117^+$than in group $CD56^+CD117^+$(42.0 months vs. 11.5 months, $p=0.002$). The OS in Group $CD56^+CD117^+$was significantly lower than those of other groups, as well ($p=0.002$) (**Fig.2**). In parallel, themortality ratewassignificantly elevated in group $CD56^+CD117^+$than in other groups ($p<0.001$) (**Table 7**).

The univariate regression analysis revealed cytogenetic risk and CD56 and CD117 co-positivity aspoor prognostic factors for OS ($p<0.001$ and $p=0.002$, respectively). Further analysis of these two factors with

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multivariate regression analysis revealed cytogenetic risk as the only factor that can significantly predictOS (Odds Ratio= -20.13, CI: -28.4—11.87, p<0.001) (Table 8).

Discussion

Our study's findingsindicatedthat CD56 and CD117 double-positive MM patients had significantlylower ORR, shorter OS, and higher mortality rates than those eitherCD56 or CD117 positive and CD56 and CD117 double-negative. While the univariate analysis revealed high cytogenetic risk and double-positive for CD56 and CD117aspoor prognostic factors for OS,multivariate analysis revealed high cytogenetic risk as the only factor that can significantly predictshorter OS in MM patients.

The prevalence of MM patients with different CD56 and CD117 immunophenotypeshasbeen investigatedin numerous studies. These studies are consistent in that they found that approximately two-thirds and one-third of MM patients wereCD56 and CD117 positive, respectively [6]. In addition, CD56 and CD117 double-negativeand CD56 and CD117 double-positive were detected in almost one-quarter to one-third of the patients with MM [1,2,14].However, there are also studies thatreported lower incidences of CD117 positive[15,16]. The discrepancies between the studies in rates of MM patients with different CD56 and CD117 levels may be attributed to the methodological variations, suggesting no significant impact on the reliabilityof 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 of these studies, Zheng et al. [1] found that CD56 negativewas a poor prognostic marker associated with increased adverse cytogenetic abnormalities and that CD56 and CD117double-negative were associated with worse clinical outcomes. Similar findings were reported in other studies [17–20]. It has been reported that CD56 positivewas significantly associated with higher ORR and OS compared to those with CD56 negative[14,21]. In a meta-analysis, Zhang et al. [18]concludedthat CD56 negativity was associated with poorer OS and progression-free survival (PFS)in MM patients. In sum, discrepancies exist between thesestudies, possibly resulting from differences in treatment regimens, diagnostic methods, survival analyses, region, and cutoff values for determining CD56 expression-based immunophenotype [11,12,18,22–26].

Contrary to the studies that found CD117 positiveas an independent poor prognostic factor for PFSin MM patients[2,3,15], some studies speculated that CD117 positive alonemight be a marker for good prognosis. Nevertheless, there are also discrepancies between the studies on survival outcomes ofMM 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 positive and CD56 and CD117 double-positive and double-negativerevealed significantly lower OS in Group $CD56^{-}CD117^{-}$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 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 thebiological characteristics of MM, renderingthe prognostic impacts of immunophenotype-based indicators controversial [5]. Therefore, furtherlarge-scale studies are neededto elucidate the prognostic valuesof immunophenotype-based indicators.

Various risk factors have been reported in the literature in patients with MM, 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 thathigher serum creatinine levels and CD56 negativewereindependent risk factors forOSin patients with MM. In comparison, we foundthat CD56 and CD117 double-positiveand high-risk cytogenetic abnormalities were associated with shorter OS, and among the two, high-risk cytogenetic abnormalities were a significantlypoorer prognostic factor.

The relationshipbetween critical cytogenetic abnormalities and survival outcomes in the context of MM has been 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 thatt (4; 14) translocations were significantly more common in CD56(-) patients. In comparison, we did not compare MM patients in different CD expression-based immunophenotype subgroups according todifferent 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.

Limitations of the Study

The study's primaryl imitation was its retrospective single-center design and relatively small sample size. The fact that we could not compare the survival outcomes of the CD expression-based immunophenotype subgroupsaccording to cytogenetic abnormalities due to the lack of a standardized classification of cytogenetic

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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. Lastly, we could not address the potential limpact of different chemotherapeutics and novel agents.

Conclusions

The study's findings revealed that MM patients with CD56 and CD117 co-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 neededto elucidate the roles of tumor-associated markers on survival outcomes ofMM patients.

Statement of Ethics

Study approval statement: This study protocol was reviewed and approved by Umraniye Education and Research Hospital local ethics committee, approval number B.10.1.TKH.4.34.H.GP.0.01/277 on 11t^{h} August 2023.
Consent to participate statement: 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.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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References

1. Zheng D, Zhu M, Li Q, Wan W, Chen Y, Jing H. Dual Negativity of CD56 and CD117 Links to Unfavorable Cytogenetic Abnormalities and Predicts Poor Prognosis in Multiple Myeloma. J Clin Med. 2022 Nov;11(21):6524.
2. Pan Y, Wang H, Tao Q, Zhang C, Yang D, Qin H, et al. Absence of both CD56 and CD117 expression on malignant plasma cells is related with a poor prognosis in patients with newly diagnosed multiple myeloma. Leuk Res. 2016 Jan;40:77–82.
3. Wang H, Zhou X, Zhu JW, Ye JN, Guo HF, Sun C. Association of CD117 and HLA-DR expression with shorter overall survival and/or progression-free survival in patients with multiple myeloma treated with bortezomib and thalidomide combination treatment without transplantation. Oncol Lett. 2018 Aug;16(5):5655–66.
4. Rawstron AC, Orfao A, Beksac M, Bezdickova L, Brooimans RA, Bumbea H, et al. Report of the European Myeloma Network on multiparametric flow cytometry in multiple myeloma and related disorders. Haematologica. 2008 Mar;93(3):431–8.
5. Skerget M, Skopec B, Zadnik V, Zontar D, Podgornik H, Rebersek K, et al. CD56 Expression Is an Important Prognostic Factor in Multiple Myeloma even with Bortezomib Induction. Acta Haematol. 2018;139(4):228–34.
6. Lebel E, Nachmias B, Pick M, Gross Even-Zohar N, Gatt ME. Understanding the Bioactivity and Prognostic Implication of Commonly Used Surface Antigens in Multiple Myeloma. J Clin Med. 2022 Mar;11(7):1809.
7. Qiu Q, Zhu P, Wang M-J, Lu X-Z, Dong Y-J, Sun Y-H, et al. [Expression of CD56 and CD19 in Patients with Newly Diagnosed Multiple Myeloma and Their Relationship with Karyotypes and Prognosis]. Zhongguo shi yan xue ye xue za zhi. 2016 Aug;24(4):1071–8.
8. Ceran F, Falay M, Dağdaş S, Özet G. Multipl Miyeloma Hastalarında Tanı Sırasında CD56 ve CD117 Ekspresyonlarının Değerlendirilmesi. Turkish J Hematol. 2017 Mar;34(3):226–32.
9. Chng WJ, Dispenzieri A, Chim C-S, Fonseca R, Goldschmidt H, Lentzsch S, et al. IMWG consensus on risk stratification in multiple myeloma. Leukemia. 2014 Feb;28(2):269–77.
10. Shin SY, Lee ST, Kim HJ, Kim SJ, Kim K, Kang ES, et al. Antigen Expression Patterns of Plasma Cell Myeloma: An Association of Cytogenetic Abnormality and International Staging System (ISS) for Myeloma. J Clin Lab Anal. 2015 Nov;29(6):505–10.
11. Hanamura I. Multiple myeloma with high-risk cytogenetics and its treatment approach. Int J Hematol. 2022 Jun;115(6):762–77.
12. Atrash S, Flahavan EM, Xu T, Ma E, Karve S, Hong WJ, et al. Treatment patterns and outcomes according to cytogenetic risk stratification in patients with multiple myeloma: a real-world analysis. Blood Cancer J. 2022 Mar;12(3):46.
13. Rajkumar SV. Multiple myeloma: 2020 update on diagnosis, risk-stratification and management. Am J

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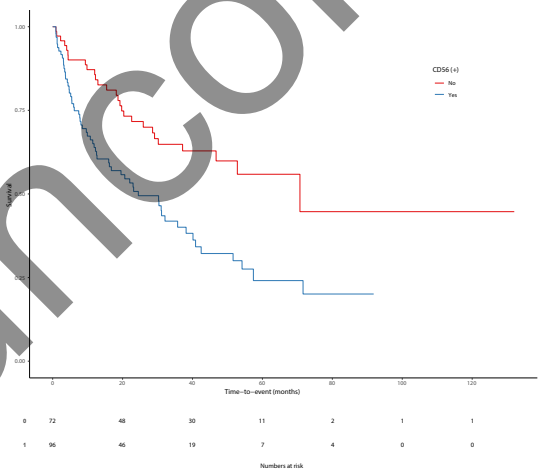
Hematol. 2020 May;95(5):548–67.
14. Shi J, Sun K, Zhu ZM, Lei PC, Liu ZW, Chen YQ, et al. Prognostic significance of CD56 and CD117 expression in patients with newly diagnosed multiple myeloma treated with bortezomib-based first-line therapy. Zhonghua Xue Ye Xue Za Zhi. 2019 Aug;40(8):693–6.
15. Mengich I, Rajput S, Malkit R, Moloo Z, Kagotho E, Lalani EN, et al. Immunophenotypic expression profile of multiple myeloma cases at a tertiary hospital in Nairobi Kenya. Front Med. 2023 May;10. DOI: 10.3389/fmed.2023.1177775
16. Shim H, Ha JH, Lee H, Sohn JY, Kim HJ, Eom HS, et al. Expression of myeloid antigen in neoplastic plasma cells is related to adverse prognosis in patients with multiple myeloma. Biomed Res Int. 2014;2014:1–8.
17. Fazio F, Lapietra G, Limongi MZ, Intoppa S, Milani ML, Piciocchi A, et al. Multiparametric Flow Cytometry in Newly Diagnosed Multiple Myeloma Patients: An Italian Monocentric Experience. Mediterr J Hematol Infect Dis. 2023 Aug;15(1):e2023047.
18. Zhang L, Huang Y, Lin Y, Zhang A, Zou R, Xu H, et al. Prognostic significance of CD56 expression in patients with multiple myeloma: a meta-analysis. Hematol (United Kingdom). 2022 Dec;27(1):122–31.
19. Chen F, Hu Y, Wang X, Fu S, Liu Z, Zhang J. Expression of CD81 and CD117 in plasma cell myeloma and the relationship to prognosis. Cancer Med. 2018 Dec;7(12):5920–7.
20. Okura M, Ida N, Yamauchi T. The clinical significance of CD49e and CD56 for multiple myeloma in the novel agents era. Med Oncol. 2020 Nov;37(11):103.
21. Yoshida T, Ri M, Kinoshita S, Narita T, Totani H, Ashour R, et al. Low expression of neural cell adhesion molecule, CD56, is associated with low efficacy of bortezomib plus dexamethasone therapy in multiple myeloma. PLoS One. 2018 May;13(5):e0196780.
22. Ocqueteau M, Orfao A, García-Sanz R, Almeida J, Gonzalez M, San Miguel JF. Expression of the CD117 antigen (C-Kit) on normal and myelomatous plasma cells. Br J Haematol. 1996 Dec;95(3):489–93.
23. Rajan AM, Rajkumar S V. Interpretation of cytogenetic results in multiple myeloma for clinical practice. Blood Cancer J. 2015 Oct;5(10):e365–e365.
24. Abduh MS. An overview of multiple myeloma: A monoclonal plasma cell malignancy's diagnosis, management, and treatment modalities. Saudi J Biol Sci. 2024 Feb;31(2):103920.
25. Maura F, Bergsagel PL. Molecular Pathogenesis of Multiple Myeloma. Hematol Oncol Clin North Am. 2024 Apr;38(2):267–79.
26. Marcon C, Simeon V, Deias P, Facchin G, Corso A, Derudas D, et al. Experts' consensus on the definition and management of high risk multiple myeloma. Front Oncol. 2023 Jan;12. DOI: 10.3389/fonc.2022.1096852
27. Gross Even-Zohar N, Pick M, Hofstetter L, Shaulov A, Nachmias B, Lebel E, et al. CD24 Is a Prognostic Marker for Multiple Myeloma Progression and Survival. J Clin Med. 2022 May;11(10):2913.
28. Pawlyn C, Morgan GJ. Evolutionary biology of high-risk multiple myeloma. Nat Rev Cancer. 2017 Sep;17(9):543–56.



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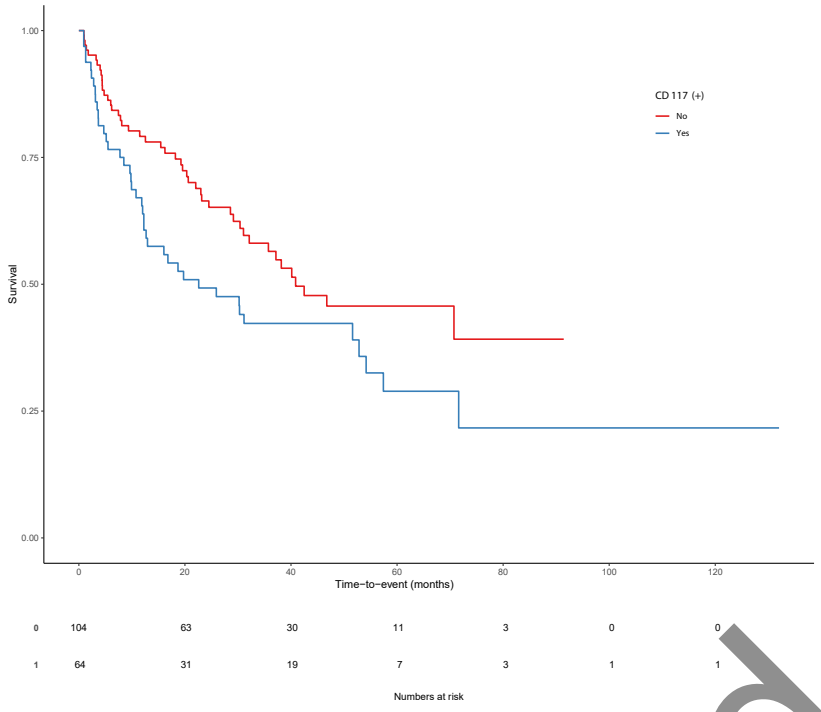

Fig. 1. The Kaplan–Meier curves for OS by patients' CD status: a) CD56 and b) CD117.

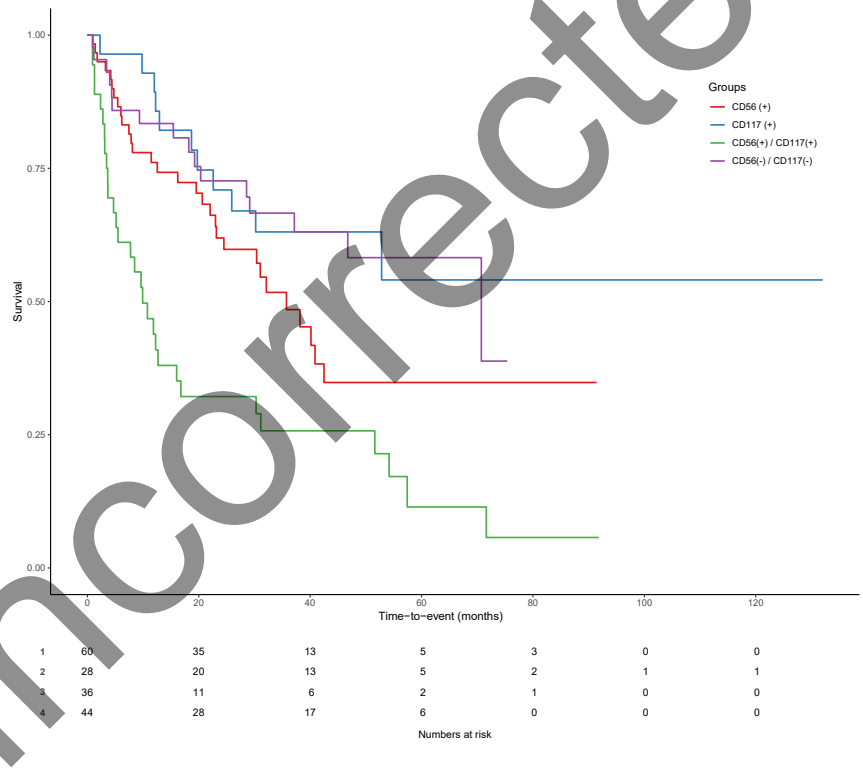

Fig. 2. The Kaplan–Meier curves for OS by groups.

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

	Group CD56 $^{+}$ (n=60)	**Group CD117^{+}** (n=28)	**Group CD56^{+}/CD117^{+}** (n=36)	**Group CD56^{-} /CD117^{-}** (n=44)	**p**
Age (*year*) †	66.1 ± 10.1	66.7 ± 10.7	68.7 ± 8.7	67.2 ± 8.5	0.618***
Age groups ‡					
<60 year	13 (21.7)	6 (21.4)	5 (13.9)	9 (20.5)	0.805*
≥60 year	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)	
Comorbidity ‡					
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 [min-max]

a, b: Different superscripts indicate statistical differences between groups in each row. There is no statistical difference between groups with the same superscripts.

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COPD: chronic obstructive pulmonary disease, CAD: coronary artery disease, ECOG-PS: Eastern Cooperative Oncology Group performance status, ISS: International Staging System.
*. Pearson Chi-Square or Fisher Freeman Halton test.
**. Kruskal Wallis-H test.
***. One-Way ANOVA test.

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

	Group CD56$^+$ (n=60)	**Group CD117$^+$** (n=28)	**Group CD56$^+$/CD117$^+$** (n=36)	**Group 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/hr*) §	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 [min-max]
*. Kruskal Wallis-H test.
**. One-Way ANOVA test.

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 Ig heavy 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)

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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)

Table 4: Cytogenetic abnormalities of the patients.

	Group CD56$^+$ (n=60)	**Group CD117$^+$** (n=28)	**Group CD56$^+$/CD117$^+$** (n=36)	**Group 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.

	Group CD56$^+$ (n=60)	**Group CD117$^+$** (n=28)	**Group CD56$^+$/CD117$^+$** (n=36)	**Group CD56$^-$ /CD117$^-$** (n=44)	**p***
First-line ‡					
Bortezomib-based	44 (73.3)	20 (71.4)	25 (69.4)	36 (81.8)	0.710
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 ‡					
Bortezomib-based	22 (44.0)	12 (44.4)	9 (42.9)	17 (41.5)	0.425

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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 ‡					
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)	**0.049**
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.

Table 6: Treatment outcomes and survival data of the 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 (*month*) §	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***
Non-survived	14 (19.4)	47 (49.0)		28 (26.9)	33 (51.6)	

‡: n (%), §: Median [min-max]
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.

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

	Group CD56 (+) (n=60)	**Group CD117 (+)** (n=28)	**Group CD56(+)/CD117 (+)** (n=36)	**Group 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 (*month*) §	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***
Non-survived	19 (31.7) [a]	5 (17.9) [a]	28 (77.8) [b]	9 (20.5) [a]	

‡: n (%), §: Median [min-max]

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.

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

	Univariate linear regression		**Multivariate linear regression**	
	Beta coefficient [CI 95%]	**p**	**Beta coefficient [CI 95%]**	**p**
Age	0.22 [-0.19 – 0.62]	0.295	-	-
Sex: *Female vs. Male*	-3.38 [-10.98 – 4.22]	0.384	-	-
ECOG-PS: *2-3 vs. 0-1*	-2.35 [-10.05 – 5.35]	0.551	-	-
ISS stage: *III vs. I-II*	-2.20 [-9.84 – 5.44]	0.573	-	-
Lactate dehydrogenase	-0.01 [-0.02 – 0.01]	0.065	-0.01 [-0.02 – 0.01]	0.167
Albumin	3.13 [-2.33 – 8.60]	0.263	-	-
Calcium	-0.51 [-3.13 – 2.11]	0.703	-	-
β2-microglobulin	0.01 [-0.52 – 0.51]	0.988	-	-
Cytogenetic abnormalities: *Present vs. absent*	-7.37 [-17.39 – 2.64]	0.151	3.35 [-6.48 – 13.19]	0.505
Cytogenetic risk: *High risk vs. standard risk*	-21.43 [-28.53 – -14.32]	**<0.001**	-20.13 [-28.4 – -11.87]	**<0.001**
CD expressions: *CD56(+)/CD117 (+) vs. others*	-14.59 [-23.61 – -5.58]	**0.002**	-4.83 [-14.12 – 4.47]	0.311
Autologous hematopoietic stem cell transplantation: *Absent vs. present*	1.16 [-6.21-8.54]	0.756	-	-
First line treatment regimens: *Non-bortezomib vs. bortezomib based*	22.15 8-5.47-49.8]	0.115	-	-

CI: confidence interval, ECOG-PS: Eastern Cooperative Oncology Group performance status, ISS: International Staging System.CI: Confidence interval.

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