



## Research Article

# Evaluation of routine laboratory parameters as clinical indicators of disease severity in multiple myeloma

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### Abstract

**Objectives:** Multiple myeloma (MM) is characterized by clonal plasma cell proliferation and significant systemic impacts. This study aimed to evaluate the relationship between routine hemogram and biochemical parameters and disease severity markers (M-protein and  $\beta$ -2 microglobulin [ $\beta$ -2M]) to identify accessible clinical indicators of tumor load at the time of diagnosis.

**Methods:** In this retrospective cross-sectional study, newly diagnosed, treatment-naïve MM patients and healthy controls were analyzed. Statistical significance was set at a threshold ( $p < 0.00125$ ) using Bonferroni correction to prevent Type I errors. Multivariable logistic regression was performed to identify independent predictors of high M-protein load ( $\geq 3\text{g/dL}$ ), and ROC analysis was used to determine the diagnostic performance of significant parameters.

**Results:** MM patients exhibited significantly lower WBC, RBC, HCT, and PLT counts, and higher BUN and CRP levels compared to controls ( $p < 0.001$ ).  $\beta$ -2M showed significant correlations with several routine parameters; however, partial correlation and multivariable regression revealed that these associations were entirely dependent on renal function. Conversely, multivariable logistic regression identified RBC count (OR=0.383,  $p=0.026$ ), eGFR, and age as significant independent predictors of high M-protein load. Notably, each  $1 \times 10^6/\mu\text{L}$  decline in RBC count was associated with a 161% increase in the risk of high disease severity. ROC analysis established an optimal RBC cut-off value of  $3.73 \times 10^6/\mu\text{L}$  (AUC: 0.695, sensitivity: 64.1%, specificity: 69.6%) for predicting high tumor load.

**Conclusion:** Routine laboratory data, particularly RBC count, serve as powerful indicators of MM severity at the time of initial diagnosis. Unlike  $\beta$ -2M, which is heavily influenced by renal status, RBC count is an independent predictor of monoclonal protein load. A baseline RBC level below  $3.73 \times 10^6/\mu\text{L}$  should alert clinicians to a potentially high tumor load, facilitating rapid triage and treatment prioritization.

**Keywords:** Beta 2-microglobulin, complete blood count, C-reactive protein, multiple myeloma, paraproteins

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Multiple myeloma (MM) is a clonal plasma cell neoplasm originating from the post-germinal lymphoid B-cell lineage [1]. It accounts for 1% of all cancers and 10% of hematological malignancies, making it the second most common hematological malignancy after lymphoma [2]. The estimated 5-year global incidence is approximately 230,000 patients. The median age of patients at diagnosis is 66–70 years. It is approximately 1.5 times more common in men than in women [3].

MM risk factors have not been fully elucidated. Factors that may pose a risk for the disease include being 65 years and older, African American race, male sex, and a family history of the disease. Patients often present with nonspecific symptoms such as weight loss, nausea/constipation, frequent urination, bone pain, weakness, and fatigue [4].

According to the International Myeloma Working Group (IMWG), the presence of hypercalcemia (serum calcium  $> 11$

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mg/dL), renal involvement (creatinine clearance < 40 mL/minute or serum creatinine > 2 mg/dL), anemia (hemoglobin < 10 g/dL or hemoglobin value > 2 g/dL below the lower limit of normal), and osteolytic bone lesions, along with M-spike peak on serum protein electrophoresis and/or monoclonal plasma cells, are necessary for diagnosis [1]. The serum free light chain (sFLC) ratio was added to the criteria in 2014 [5]. The International Staging System (ISS) uses albumin and  $\beta$ -2 microglobulin ( $\beta$ -2M) levels to determine the risk and stage of MM [6].

MM can be confused with different diseases in terms of the age of occurrence, nonspecific symptoms, and laboratory results; therefore, delays in diagnosis and treatment can be experienced. Depending on the stage at the time of diagnosis, the 5-year (2015–2021) relative survival rates are approximately 60% [7].

Considering that clinical evaluation at the time of presentation and treatment significantly affect MM prognosis, it is thought that rapid assessment based on laboratory data is important. In this study, we aimed to examine the relationships of routine hemogram and certain biochemistry parameters of MM patients with M-protein and  $\beta$ -2M levels and to emphasize the potential value of these parameters as clinical indicators reflecting disease severity in MM.

## Materials and Methods

This retrospective cross-sectional analytical study included patients diagnosed with MM who presented to Kayseri City Training and Research Hospital between July 1, 2022, and December 31, 2022, for follow-up and treatment, and age- and sex-matched healthy participants. Patients with cardiovascular and autoimmune diseases, severe liver or renal failure, other malignancies/infections, and pregnancy were excluded. Laboratory data for all patients included in the study were obtained from baseline values at the time of initial diagnosis, prior to the initiation of any plasma cell-targeted therapy. Demographic data of the participants were obtained from the laboratory information system.

Biochemistry analyses were performed using Cobas 8000 (Roche Diagnostics®, Mannheim, Germany), and hemogram analyses were performed using Sysmex XN-1000® (Sysmex, Kobe, Japan) autoanalyzers with original kits and reagents. Protein hydra gel electrophoresis (Hydras Sebia®, USA) was used for serum protein electrophoresis. In the determination of serum protein electrophoresis (SPEP) fractions and the amount of M-protein, the perpendicular drop method was used while calculating the peak area in the electrophoresis pattern to provide consistent measurement at different protein concentration levels. All measurements were performed in accordance with the standard protocols of the laboratory. No restriction was made regarding the location of the M-protein peak; patients with monoclonal peaks in both the gamma and beta regions—although the latter constituted a small part of the total cohort—were included in the study to reflect biological diversity. Especially in the measurement of

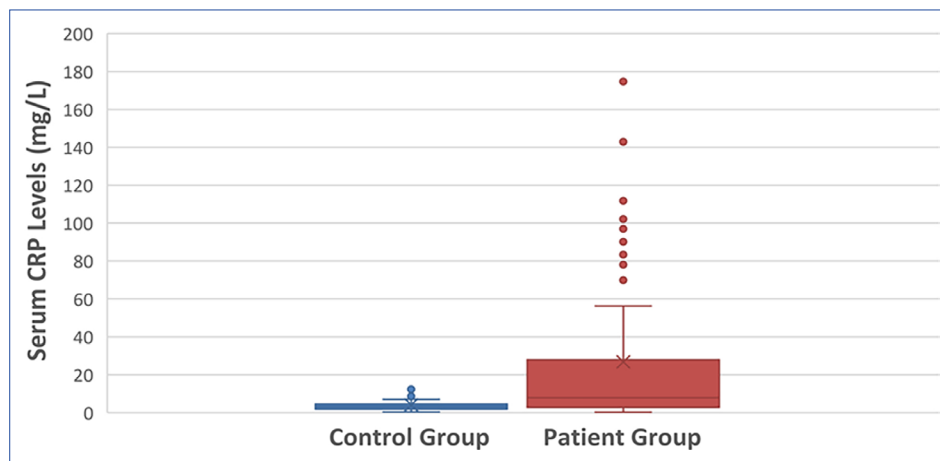
M-proteins located in the beta region, the perpendicular drop method was meticulously applied to minimize artifacts that could arise from normal beta-1 and beta-2 fractions and to ensure accurate quantitation. All monoclonal peaks were distinguished from other protein fractions by confirmation with immunofixation electrophoresis (IFE).

The studied biochemistry parameters were glucose, creatinine, eGFR, BUN, uric acid, cholesterol, LDL-cholesterol (direct), HDL-cholesterol, triglyceride, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), amylase, lipase, total bilirubin, direct bilirubin, calcium ( $\text{Ca}^{2+}$ ), sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ), magnesium ( $\text{Mg}^{2+}$ ), phosphorus, iron, free iron binding capacity (FIBC), transferrin saturation, creatine kinase (CK), creatine kinase-MB (CK-MB), rheumatoid factor (RF), and C-reactive protein (CRP). Furthermore, IgG, IgM, IgA,  $\beta$ -2M, M-protein, kappa, and lambda light chain levels of the participants were also examined. Biochemistry and hemogram parameters were statistically compared.

The minimum sample size required for each group was determined using the G\*Power 3.1.9.4® program based on an independent groups t-test analysis with 5% type 1 error ( $\alpha$ ), 95% test power ( $1-\beta$ ), and an effect size of  $d=0.8$  [8]. Microsoft Office Excel 2016 was used to compile the data. Statistical analysis was performed using SPSS 22.0® (Statistics Package for Social Sciences). For group comparisons and correlation analyses, only patients with complete data for the relevant parameters were included. The Shapiro–Wilk normality test was used to examine sample distributions. While the independent groups t-test was used to compare parametric data, the Mann–Whitney U test was used to compare non-parametric data. Pearson's and Spearman's correlation tests were used in correlation analyses depending on the homogeneity status.

In the study, to eliminate the risk of Type I error that could arise from the comparison of approximately 40 parameters, Bonferroni correction was applied from the very beginning. In this context, while comparing the initial stage routine parameters of the patient and control groups, the significance threshold was redefined as  $p < 0.00125$  ( $0.05/40$ ). Only parameters falling below this strict threshold value were considered statistically significant. The correlation of parameters showing a significant difference between groups with M-protein and  $\beta$ -2M, which are indicators of disease severity in the MM patient group, was examined. Again, to minimize the risk of Type I error that multiple comparisons could cause, Bonferroni correction was applied separately for both independent variables (M-protein and  $\beta$ -2M). Accordingly, the significance threshold was determined as  $p < 0.0083$  ( $0.05/6$ ) for 6 parameters associated with M-protein, and as  $p < 0.0062$  ( $0.05/8$ ) for 8 parameters associated with  $\beta$ -2M.

Multivariable logistic regression analysis was performed to identify the independent predictors of high M-protein load ( $\geq 3\text{g/dL}$ ) at the time of diagnosis. Multicollinearity among the



**Figure 1.** Comparison of serum CRP level between patient and control group.

CRP: C-reactive protein

independent variables included in the model was assessed by calculating the variance inflation factor (VIF); a VIF value of  $<2.5$  was considered as the absence of multicollinearity. The goodness-of-fit of the model to the data was evaluated using the Hosmer–Lemeshow test. Receiver operating characteristic (ROC) curve analysis was conducted to determine the diagnostic performance and optimal cut-off point for the red blood cell count (RBC) parameter, which was found to be significant in the logistic regression analysis. The optimal cut-off value was established by calculating the Youden index (sensitivity+specificity–1) to maximize the sum of sensitivity and specificity. Area under the curve (AUC) values are presented alongside their respective 95% confidence intervals (CI).

Partial correlation analysis was performed to evaluate the independence of the relationships between  $\beta$ -2M and routine laboratory parameters (RBC, HCT, HGB, BUN, and CRP) from renal function, with eGFR defined as a control variable. Additionally, to test the independent effect of routine parameters in predicting  $\beta$ -2M levels, a multivariable regression analysis was conducted, in which  $\beta$ -2M was the dependent variable, and RBC, HCT, HGB, eGFR, BUN, and CRP were the independent variables. The study was conducted in accordance with the Helsinki Declaration (as revised in 2013) and received ethical committee approval from Kayseri City Training and Research Hospital (Date: 25.03.2025, Decision No: 376). Written informed consent was obtained from the patients.

## Results

Data from 94 patients and 51 healthy controls were included in the study. A statistically significant difference was observed between the patient and control groups in white blood cell count (WBC), absolute basophil count, RBC, hematocrit (HCT), platelet count (PLT), BUN, and  $\text{Ca}^{2+}$  parameters ( $p < 0.001$ ). As a result of the analyses performed, while the median WBC value of the patient group was 5.74 (2.13–16.62), this value was determined as 6.85 (3.98–11.98) in the control group. The median value of CRP in the patient group was 7.85 (0.3–175)

mg/L, while it was determined as 3.10 (0.4–14.3) mg/L in the control group (Fig. 1). Despite the applied Bonferroni correction ( $p < 0.00125$ ), it was observed that both parameters maintained their statistical significance between the groups ( $p < 0.001$ ). Other laboratory and demographic data of the study groups are shown in Table 1.

A negative correlation was found between the RBC, HCT, and PLT parameters, which were different in MM patients compared to the control group, and M-protein levels.  $\beta$ -2M protein levels showed a negative correlation with RBC, hemoglobin (HGB), HCT, and eGFR, and a positive correlation with BUN and CRP. Correlation analyses showing all other relationships are summarized in Table 2. In serum free light chain (sFLC) analyses, no statistically significant correlation was found between free kappa, free lambda, and kappa/lambda ratio and routine hemogram parameters (RBC and HCT) ( $p > 0.05$ ) (Table 3).

Patients were divided into two groups based on M-protein levels (Group A:  $<3\text{g/dL}$  and Group B:  $\geq 3\text{g/dL}$ ) [9]. Absolute basophil count showed a negative correlation with Group B M-protein levels, whereas a similar relationship was not observed in Group A (Fig. 2a). A negative correlation was observed between RBC and HCT levels, which were significantly lower in MM patients than in the control group, and Group A M-protein levels. Conversely, no similar correlation was detected in Group B (Fig. 2b, c). While albumin levels were found to be negatively correlated with high levels of M-protein, a similar relationship was not found with low levels ( $p = 0.04$ ,  $R_{ho} = -0.652$ ) (Fig. 3).

Multivariable logistic regression analysis, including age, sex, eGFR, and RBC parameters, was performed to identify the independent risk predictors of high M-protein load ( $\geq 3\text{g/dL}$ ) at the time of diagnosis. In the multicollinearity analysis conducted to evaluate the relationship between independent variables, the VIF values for RBC and eGFR were found to be below 2.5, confirming that the variables in the model functioned independently. Furthermore, the Hosmer–Lemeshow goodness-of-fit test demonstrated that the model was well

**Table 1. Laboratory and demographic mean (min-max) or mean±SD of the study groups**

Parameters	Patient (n=94*)	Control (n=51)	p	Bonferroni correction
Age (years)	65 (34–83)	55 (42–75)	>0.05	Insignificant
Gender / sex	54 M / 40 F	22 M / 29 F	>0.05	Insignificant
WBC (10 <sup>3</sup> /μL)	5.74 (2.13–16.62)	6.85 (3.98–11.98)	0.001	Significant
BASOPHIL (10 <sup>3</sup> /μL)	0.02 (0.00–0.12)	0.025 (0.01–0.15)	0.001	Significant
NEUTROPHIL (10 <sup>3</sup> /μL)	3.07 (0.22–10.38)	4.73 (2.11–30.14)	0.014	Insignificant
LYMPHOCYTE (10 <sup>3</sup> /μL)	1.47 (0.21–4.91)	1.57 (1.49–2.24)	0.001	Significant
RBC (10 <sup>6</sup> /μL)	3.83±0.81	4.36±0.81	0.001	Significant
HGB (g/dL)	11.42±2.22	12.22±2.06	0.001	Significant
HCT (%)	34.02±6.21	36.72±6.78	0.001	Significant
MCV (fL)	89.9 (68–107)	84.2 (81–88)	0.001	Significant
PLT (10 <sup>3</sup> /μL)	180.000 (20.000–645.000)	272.000 (197.000–445.000)	0.001	Significant
RDW-SD (fL)	50 (37–71)	43 (36–50)	0.001	Significant
RDW-CV (%)	15 (12–24)	14 (12–17)	0.001	Significant
BUN (mg/dL)	15.5 (5–64)	10 (6–19)	0.001	Significant
Creatinin (mg/dL)	1.36±1.37	0.70±0.21	0.009	Insignificant
eGFR (mL/dk/1.73 m <sup>2</sup> )	73±30	96±17	0.001	Significant
Ca <sup>2+</sup> (mg/dL)	9.0 (6.6–11.5)	9.1 (7.9–9.6)	0.001	Significant
Na <sup>+</sup> (mmol/L)	138 (126–146)	140 (136–142)	0.001	Significant
K <sup>+</sup> (mmol/L)	4.2 (2.7–5.8)	4.25 (3.4–5.3)	0.001	Significant
CRP (mg/L)	7.85 (0.3–175)	3.10 (0.4–14.3)	0.001	Significant

\*: The numbers represent the total cohort (n=94 patients). Due to the retrospective nature of the study, certain laboratory parameters were not available for all individuals. The actual sample size for some variables may be slightly lower than the total cohort size. BUN: Blood urea nitrogen; CRP: C-reactive protein; eGFR: Estimated glomerular filtration rate; HCT: Hematocrit; HGB: Hemoglobin; MCV: Mean corpuscular volume; PLT: Platelet; RBC: Red blood cell; RDW-CV: Red cell distribution width- coefficient of variation; RDW-SD: Red cell distribution width-standart deviation; WBC: White cell count; p: Probability value. Data are presented as mean and median based on their homogeneity status. Bonferroni correction was applied to the p-values to prevent Type I errors that may arise from multiple comparisons (p<0.00125).

**Table 2. Correlations of M-Protein and β-2 microglobulin levels with parameters differing from the control group in patients**

	Parameters	Correlation coefficient (r/R <sub>ho</sub> )	Test type	p	Bonferroni correction
M-protein (g/dL)	RBC (10 <sup>6</sup> /μL)	-0.525	Pearson (r)	0.001	Significant
	HGB (g/dL)	-0.462	Pearson (r)	0.001	Significant
	HCT (%)	-0.496	Pearson (r)	0.001	Significant
	PLT (10 <sup>3</sup> /μL)	-0.275	Spearman (R <sub>ho</sub> )	0.042	Insignificant
	BASOPHIL (10 <sup>3</sup> /μL)	-0.377	Spearman (R <sub>ho</sub> )	0.001	Significant
	RDW-SD (fL)	0.272	Spearman (R <sub>ho</sub> )	0.045	Insignificant
β-2microglobulin (mg/L)	RBC (10 <sup>6</sup> /μL)	-0.415	Spearman (R <sub>ho</sub> )	0.001	Significant
	HGB (g/dL)	-0.459	Spearman (R <sub>ho</sub> )	0.001	Significant
	HCT (%)	-0.440	Spearman (R <sub>ho</sub> )	0.001	Significant
	eGFR (mL/dk/1.73 m <sup>2</sup> )	-0.609	Spearman (R <sub>ho</sub> )	0.001	Significant
	RDW-SD (fL)	0.386	Spearman (R <sub>ho</sub> )	0.01	Insignificant
	RDW-CV (%)	0.279	Spearman (R <sub>ho</sub> )	0.016	Insignificant
	BUN (mg/dL)	0.526	Spearman (R <sub>ho</sub> )	0.001	Significant
	CRP (mg/L)	0.444	Spearman (R <sub>ho</sub> )	0.001	Significant

The normality of data distribution was assessed using the Shapiro-Wilk test. Correlation analyses were performed using Pearson's (r) for normally distributed parameters and Spearman's (R<sub>ho</sub>) for non-normally distributed variables. To mitigate the risk of Type I errors arising from multiple comparisons, a Bonferroni correction was applied. Specifically, the correction was executed independently for each dependent variable (M-protein and beta-2-microglobulin). Accordingly, the significance threshold was adjusted to p<0.0083 for the 6 parameters associated with M-protein, and to p<0.0062 for the 8 parameters associated with beta-2-microglobulin. \*Note: Correlation analyses were conducted only on patients with complete data for the specific parameters. Due to the retrospective nature of the study, the sample size for each parameter may vary based on availability of data in the records. RBC: Red blood cell count; HGB: Hemoglobin; HCT: Hematocrit; PLT: Platelet; RDW-SD: Red cell distribution width-standart deviation; RDW-CV: Red cell distribution width- coefficient of variation.

fitted to the data (p>0.05). The analysis revealed that among the variables included in the model, RBC (β=-0.959; S.E.=0.430; p=0.026; OR=0.383; 95% CI:0.165–0.891), eGFR (β=-0.041;

S.E.=0.014; p=0.004; OR=0.960; 95% CI:0.934–0.987), and age (β=0.085; S.E.=0.035; p=0.015; OR=1.088; 95% CI:1.016–1.165) were determined to be significant independent predictors of

**Table 3. Correlation of free light chain (sFLC) parameters with erythroid indices RBC ( $10^6/\mu\text{L}$ ) and HCT (%)**

Parameters	Median (min-max)	HCT ( $R_{ho}/p$ )	RBC ( $R_{ho}/p$ )
Kappa ( $\kappa$ ) (mg/L)	2.84 (0.53–33.55)	-0.076/0.510	-0.028/0.810
Lambda ( $\lambda$ ) (mg/L)	1.51 (0.22–15.61)	-0.024/0.832	-0.019/0.867
$\kappa/\lambda$ ratio	1.88 (0.05–139.79)	-0.011/0.925	-0.001/0.996

Since the data did not follow a normal distribution, Spearman's correlation analysis was performed. The results indicated that sFLC levels, and specifically the kappa/lambda ratio, which holds high diagnostic value, did not show a significant correlation with HCT and RBC levels ( $p>0.05$ ). This finding quantitatively supports our results that the relationship between M-protein levels and hemogram parameters is more prominent compared to that of sFLC. RBC: Red blood cell count; HCT: Hematocrit.

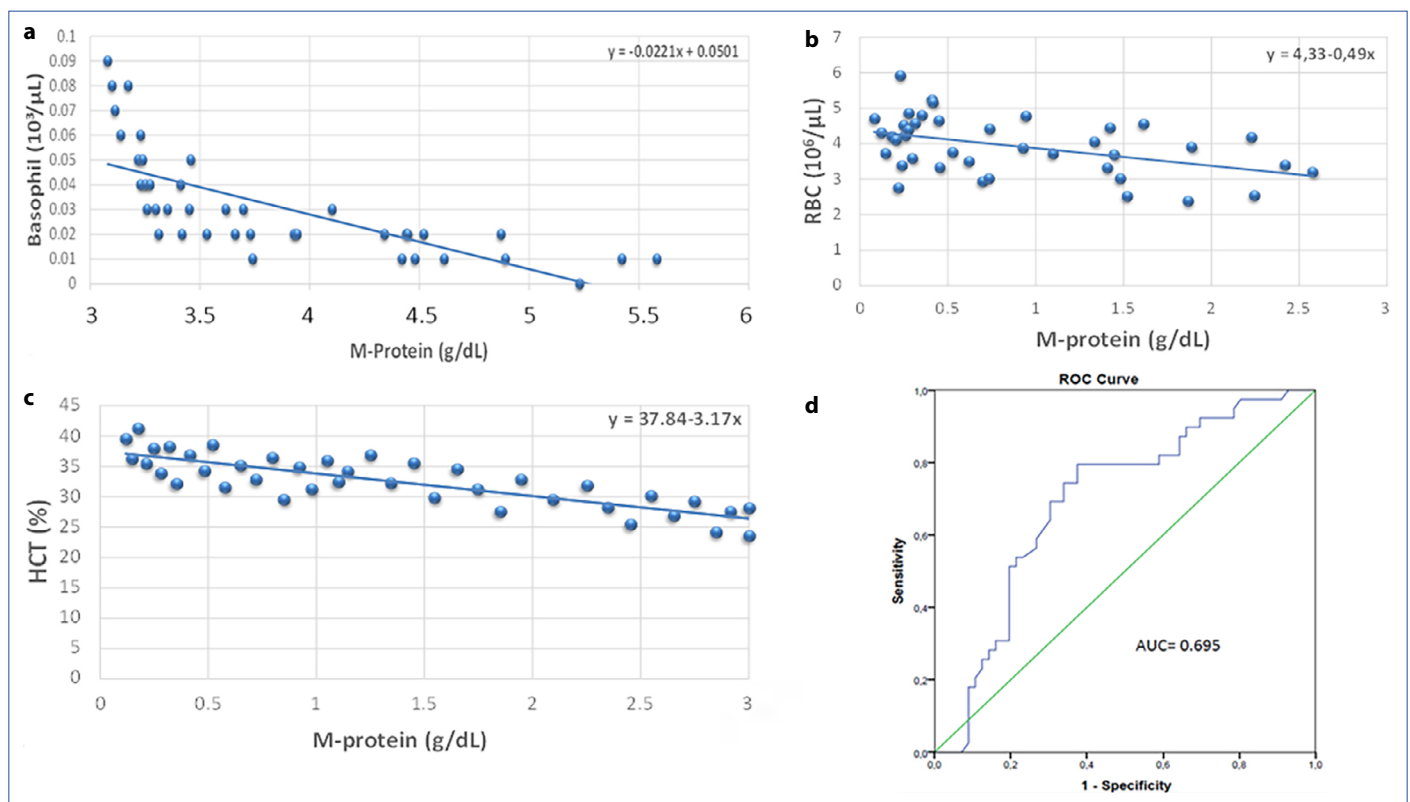
high M-protein load. The sex variable did not show a statistically significant independent effect in the model ( $p=0.07$ ).

The diagnostic performance of the RBC parameter, which was proven to be an independent predictor in the logistic regression model, was further evaluated using ROC analysis for predicting high M-protein load ( $\geq 3\text{g/dL}$ ) at diagnosis. The analysis yielded an area under the curve (AUC) value of 0.695 for the RBC level, which was found to be statistically significant (S.E.=0.055;  $p=0.001$ ; 95% CI:0.587–0.803) (Fig. 2d). The RBC optimal cut-off value providing the highest accuracy for predicting high M-protein load was established at  $3.73 \times 10^6/\mu\text{L}$ . At this threshold, the sensitivity and specificity

of the RBC parameter in predicting high disease severity were calculated as 64.1% and 69.6%, respectively.

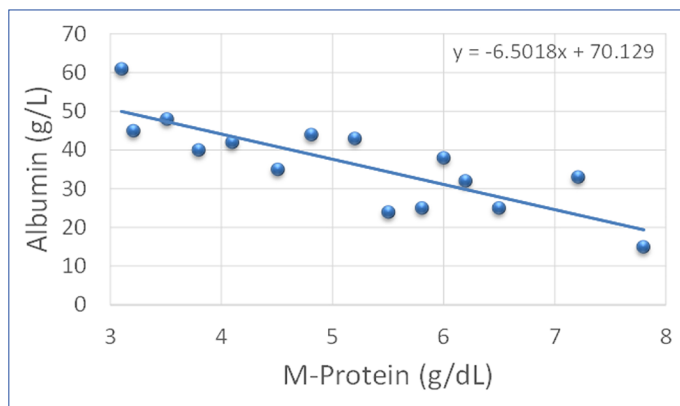
The correlations between  $\beta$ -2M and significant parameters were re-examined using partial correlation analysis, adjusting for the effect of eGFR. Once eGFR was controlled, the previously significant relationships between  $\beta$ -2M and other parameters were found to lose their statistical significance ( $p>0.05$ ).

In the multivariable regression analysis conducted to identify the independent factors influencing  $\beta$ -2M levels, eGFR was determined to be the only statistically significant independent predictor of  $\beta$ -2M levels ( $p<0.05$ ). No independent effect on  $\beta$ -2M levels was observed for the other parameters ( $p>0.05$ ).



**Figure 2.** Correlation of hematological parameters with M-protein levels and predictive performance of RBC for high disease severity. (a) Scatter plot demonstrating the correlation between Group B ( $\geq 3\text{g/dL}$ ) M-protein concentrations and basophil counts. A statistically significant negative correlation ( $n=40$ , due to missing CBC data in some patients,  $R_{ho}=-0.43$ ,  $p<0.05$ ) was observed. (b) Relationship of Group A ( $<3\text{g/dL}$ ) M-protein levels with RBC ( $n=41$ , due to missing CBC data in some patients,  $p=0.01$ ) and (c) HCT ( $n=41$ , due to missing CBC data in some patients,  $p=0.048$ ). (d) ROC curve analysis of RBC for predicting high disease severity (M-protein  $\geq 3\text{g/dL}$ ) (AUC: 0.695, 95% CI: 0.587–0.803; Cut-off Value:  $3.73 \times 10^6/\mu\text{L}$ , Sensitivity: 64.1% and Specificity: 69.6%).

RBC: Red blood cell; CBC: Complete blood count; HCT: Hematocrit; ROC: Receiver operating characteristic.



**Figure 3.** Correlation between serum M-protein and albumin levels in patients with high M-protein load ( $\geq 3$  g/dL) ( $n=15$ , due to missing albumin data in some patients).

## Discussion

The occurrence of MM at an advanced age and the non-specific nature of symptoms can lead to complexity in the clinical evaluation at the time of presentation, causing the extent of the disease to be overlooked. This study demonstrates how routine laboratory data, frequently used by physicians in primary care, exhibit a correlation with M-protein and  $\beta$ -2M, which serve as clinical indicators of disease severity. To our knowledge, there is no other study in the literature that analyzes our study parameters by classifying according to M-protein levels.

The clinical management at any stage, from diagnosis to treatment, is crucial for clinical outcomes. Therefore, easily and quickly accessible laboratory parameters are valuable as indicators of the systemic impact of MM. In this study, a statistically significant correlation was found between HCT and RBC levels, which were significantly lower in MM patients compared to the control group, and M-protein and  $\beta$ -2M levels, reflecting an association with the disease severity at the time of presentation.

In MM, current prognosis and staging are conducted via the Revised International Staging System (R-ISS), which includes serum albumin, LDH, and  $\beta$ -2M levels, as well as cytogenetic data [10]. The significant correlations we identified in our study between  $\beta$ -2M and routine laboratory parameters provide important evidence that  $\beta$ -2M elevation, a fundamental component of R-ISS, can be predicted through routine hemogram and biochemistry tests even before advanced staging investigations are performed. Although advanced tests such as cytogenetic analyses are an integral part of R-ISS, the data presented by our study support that, especially in primary health care services with limited resources, routine hemogram and biochemistry findings can be critical preliminary indicators in predicting  $\beta$ -2M elevation—one of the R-ISS components—and thus in referring the patient to an advanced center for accurate staging. In addition, our analyses demonstrated that the variation of  $\beta$ -2M in our cohort

was substantially influenced by renal function. While eGFR emerged as a significant independent predictor ( $p < 0.05$ ), it is noteworthy that the associations between  $\beta$ -2M and other routine parameters vanished once renal function was controlled via partial correlation ( $p > 0.05$ ). This phenomenon may limit the capacity of  $\beta$ -2M to independently reflect myeloma biology, potentially creating a risk of misleading disease severity assessment during clinical staging. While we accept the prognostic value of  $\beta$ -2M, our findings statistically emphasize the necessity for complementary parameters—such as RBC—that are more stable and unaffected by renal function during the initial evaluation process.

M-protein, a monoclonal immunoglobulin secreted by plasma cells, is a quantitative parameter indicating clonal proliferation and disease progression in MM [11]. However, it is reported in the literature that M-protein levels may be too low to be detected by electrophoresis in some MM subgroups. Dispenzieri et al. [12] reported that M-spike was not observed or was faint on electrophoresis in patients with non-secretory MM, light chain MM, and IgD MM, and that serum free light chain level should be used for diagnosis in these groups. Blade and Kyle [13] showed that light chain measurement has high sensitivity in the diagnosis of non-secretory MM and IgD MM. Similarly, Mead et al. [14] compared the sensitivity of serum/urine protein electrophoresis with serum light chain levels in MM patients and found that serum light chain measurement was more sensitive than serum/urine protein electrophoresis. In our study, a significant relationship was found between M-protein levels and routine hemogram parameters, while the correlation between serum light chain levels and routine hemogram parameters was weaker. These findings indicate that the hematological anomalies in our study exhibit a closer relationship with the intact M-protein level rather than the free light chain load, further supporting the association with disease severity. Additionally, it is known in the pathogenesis of MM that renal involvement is a clinical manifestation of systemic disease progression [15]. The reason why M-protein and serum light chain levels show correlations of different intensities may be due to the fact that these markers reflect different aspects of the disease severity at the time of presentation. Indeed, data of patients at the time of admission were included in our study regardless of the clinical stage, representing a broad spectrum of tumor load.

There are studies where hematological parameters are used to evaluate suspicious clinical situations that may require further investigation for paraproteinemia. Li et al. [16], in a case-control study, examined hemogram parameters including RBC, PLT, and WBC to assess hemostatic imbalance in previously diagnosed MM patients and found that WBC, RBC, PLT, HCT, and HGB levels were lower, while IFR, RDW-CV/SD, and MPV levels were higher in the patient group compared to the healthy control group. Unlike this study, no difference was observed between the groups in terms of IFR and MPV parameters in our study. However, our other findings were similar. When IFR and

MPV parameters were evaluated according to M-protein levels, no difference was observed between Group A and Group B. The patients in the study by Li et al. [16] were individuals followed up with MM diagnosis and regularly taking medication. IFR and MPV parameters may have differed depending on the stage of the disease and the medications used. In our study, which included a higher number of patients, the fact that the patients were evaluated with their data at the time of initial presentation and the anemia picture had not yet fully developed may explain why this difference was not observed. Røllum-Larsen et al. [17] investigated the relationship between WBC count and paraproteinemia in patients with monoclonal gammopathy and found a decrease in WBC count in the early stage of the disease and an increase in the advanced stage. In the same study, the frequency of monoclonal gammopathy was found to be higher in patients with low WBC counts, and the risk of paraproteinemia was significantly higher in men compared to women. In our study, M-protein levels, both high and low, were found not to show a significant difference between male and female sexes. Rollum-Larsen et al. [17] found that when they grouped patients according to WBC count, the odds ratio for monoclonal gammopathy risk was 1.61 times higher in the group with low WBC counts. In contrast, in our study, patients were divided into two groups based on M-protein levels, and the correlation of both the percentage and absolute values of WBC count and its subparameters with M-protein levels was examined. Absolute basophil and neutrophil counts showed a negative correlation with Group B M-protein levels, while a similar relationship was not observed in Group A. No significant correlation was found between WBC count and both Group A and Group B M-protein levels. Also, differently in our study, the WBC count in Group B was found to be lower than in Group A. These variations in WBC levels may reflect the balance between cytokine-mediated inflammatory signaling and the progressive infiltration of pathological plasma cells within the bone marrow. According to our findings, changes in WBC levels do not provide additional information regarding the clinical status of MM, but changes in WBC subgroups seem to be more relevant markers reflecting the systemic impact and disease severity.

In the literature, it is known that the strong relationship between anemia and malignancy incidence becomes more pronounced with age [18]. The fact that the patient and control groups in our study exhibited a similar distribution in terms of age ( $p > 0.05$ ) provided an advantage when evaluating our results. The absence of age differences between the groups supports our opinion that the hematological and biochemical changes we detected may be primarily related to the pathological effects of MM, rather than natural processes related to aging. This situation provides a reasonable basis for the parameters we examined to be evaluated as clinical indicators reflecting the systemic impact and disease severity of MM.

Kyle et al. [19] reported that anemia, generally normocytic-normochromic, is common in MM patients. Anemia is

suggested to occur due to reduced erythropoietin levels associated with kidney disease, suppression of erythropoiesis by cancer cells, and rouleau formation of erythrocytes with abnormal immunoglobulins in approximately 80% of cases. In the same study, it was stated that WBC and PLT counts were normal, and RBC and HGB values were low in the majority of patients. Similarly, in our study, RBC and HGB values were found to be significantly lower in MM patients compared to the control group. Additionally, RBC and HCT levels were observed to show a significant correlation with Group A M-protein levels. The fact that RBC and HCT parameters showed a negative correlation with low levels of M-protein but did not show any relationship with high M-protein levels makes the hemogram analysis important, especially in patients presenting with a lower tumor load. Furthermore, in our study, WBC and PLT counts were lower compared to the control group.

In another study, similar to ours, decreased RBC levels were found in MM patients, but differently, no change was observed in mean corpuscular volume (MCV) [20]. In our study, MCV levels in the patient group were significantly higher than in the control group. This increase in MCV was significantly positively correlated with Group A M-protein levels, while a similar relationship was not observed with Group B M-protein levels. In our study, the significant decrease in RBC, HGB, and HCT levels in MM patients is consistent with the literature, reflecting the suppression of erythropoiesis by malignant plasma cells. Multivariable logistic regression analysis revealed that the RBC count independently predicted high M-protein load ( $\geq 3\text{g/dL}$ ) at the time of diagnosis (OR=0.383;  $p=0.026$ ), after adjusting for critical factors such as age and renal function (eGFR). While anemia in MM can arise from cytokine-mediated suppression of erythropoiesis and bone marrow infiltration, our findings reflect that a decrease in RBC count is not merely a secondary complication. Instead, it serves as an indicator of disease severity correlated with tumor mass and monoclonal protein synthesis capacity. To our knowledge, while the association between anemia and tumor load in MM is well-documented via hemoglobin levels, our study is among the first to define a specific RBC cut-off value ( $3.73 \times 10^6/\mu\text{L}$ ) as an independent predictor of high M-protein load. The analysis reveals that each  $1 \times 10^6/\mu\text{L}$  decline in RBC count increases the risk of presenting with a high systemic tumor load by 161%. This suggests that the depletion of the erythroid lineage is not just a side effect, but a direct reflection of the malignant plasma cell expansion within the bone marrow niche.

The RBC cut-off value of  $3.73 \times 10^6/\mu\text{L}$  is a specific finding derived from our cohort. This value suggests that risk estimation in newly diagnosed myeloma patients can be performed using routine and rapid tests, such as a complete blood count. Low RBC levels detected at diagnosis should alert the clinician not only to the presence of anemia but also to the potentially heavy monoclonal protein load. Patients whose RBC levels fall below this threshold carry a higher risk of high tumor

load and may be prioritized for further diagnostic workup and treatment scheduling. This simple yet effective approach provides clinicians with valuable time while awaiting invasive bone marrow biopsy results and establishes a strategic triage framework that could improve prognosis in patient management. Nonetheless, we suggest that each center should define its own optimal cut-off values in light of their specific patient profiles and laboratory standards.

The macrocytosis observed in our study could be attributed to various factors: nutritional deficits (B12 or folate), compensatory reticulocytosis due to bone marrow stress, or alterations in the marrow microenvironment directly caused by the plasma cell dyscrasia.

Kyle et al. [19] examined the biochemistry parameters of MM patients and observed that serum BUN, creatinine, LDH, and uric acid levels could increase, and the prevalence of hypercalcemia was 13%. Similarly, another study evaluated the changes in biochemistry parameters according to the stages of MM patients and found a significant increase in serum BUN, creatinine, and uric acid levels and a decrease in albumin levels with the progression of the disease stage. In the same study, no statistically significant difference was found in calcium levels between stages [21]. In another study, the prevalence of hypercalcemia in MM patients was reported as 47.4% [22]. In our study, the prevalence of hypercalcemia was found to be quite low at 6.55% compared to the literature. In fact, calcium levels were significantly lower in the patient group compared to the control group. So, the number of biochemistry parameters evaluated in these studies was an average of 10, approximately 40 biochemistry parameters were compared simultaneously in our study [19, 21, 22]. When we evaluated according to M-protein levels, no increase in calcium level was observed in Group B, contrary to expectations. High calcium levels occur in MM due to bone involvement [1]. Indeed, Kyle et al. [19] studies reported that 79% of the participants had radiological involvement. In other studies, when the symptoms of the participants were examined, bone pain complaints were observed with a frequency ranging from 31.6% to 50%, and this was reported to be due to bone involvement [21, 22]. Due to the retrospective design of our study and the reliance on routine clinical records at the time of diagnosis, a complete R-ISS staging based on cytogenetic (FISH) data could not be performed for all patients. However, the biochemical profile of our cohort—characterized by a low prevalence of hypercalcemia and the observed mean eGFR values—reflects a clinical spectrum where the majority of our patients had not yet developed the CRAB symptoms. This profile is considered the primary factor explaining the lower rates of hypercalcemia in our study population. In this context, the absence of extensive bone involvement that would cause hypercalcemia in these patients may explain this low level at the time of diagnosis.

Consistent with literature findings, creatinine and BUN levels were higher in the patient group compared to the

control group, but no difference was observed between uric acid and LDH levels. These two parameters are markers that reflect cell destruction and systemic tumor activity. Cell destruction increases with the increase in tumor size in tumoral tissues. Therefore, an increase in these two parameters is expected in patients with a high disease severity. Indeed, one of the studies mentioned reported that 38% of the participants were Stage 3 MM patients [21]. The lack of a difference in LDH and uric acid levels in our study can be explained by the fact that patients were evaluated at the time of initial presentation.

In our study, albumin levels were significantly negatively correlated with high levels of M-protein, while a similar relationship was not found with low levels. It is known that renal losses increase with kidney damage [15].

The relationship between CRP levels and solid organ malignancies has been investigated, but there are a limited number of studies examining its relationship with MM. Serum CRP levels can increase in many diseases, including malignancy. Zeng et al. [23], in a case-control study, examined the relationship between common types of malignancy and CRP levels and reported a positive relationship between malignancy and CRP levels. Similar relationships have also been reported in a recent study [24]. CRP levels have been shown to be a prospective biomarker in different types of malignancies [25, 26]. In a cohort study, several parameters including CRP were examined as potential biomarkers in the etiology of MM, and no significant relationship was demonstrated between CRP concentration and the disease during the follow-up period [27]. In our study, the median CRP value detected in MM patients (7.85 mg/L) was significantly higher than the control group (3.10 mg/L), reflecting the inflammatory process in which the disease progresses. The fact that the upper limit of CRP in the patient group reaches high values such as 175 mg/L demonstrates the severity of secondary inflammatory responses accompanying the disease severity in some cases. The maintenance of the significance of our findings even at a threshold value of  $p < 0.00125$  offers the idea that routine inflammatory markers could be valuable indicators reflecting the systemic impact of MM. The inflammatory response is known to occur due to the immune system's reaction to eliminate harmful stimuli, including damaged cells, pathogens, and tumor cells. In addition, tumoral tissue can secrete pro-inflammatory cytokines. Therefore, cancer is an inflammatory process. Our findings also suggest that inflammatory parameters can be associated with MM disease from the time of clinical presentation. Inflammatory parameters are used by clinicians to evaluate underlying chronic diseases, including malignancy. Guidelines recommend CRP measurement among the laboratory tests suggested for MM evaluation [28]. A recent study has revealed that a high CRP level is not only an inflammatory indicator but is also associated with disease severity in MM [29]. In line with these findings, our study shows that CRP levels are also a clinical parameter that reflects the dis-

ease severity of MM. In our study, although CRP levels were significantly higher in MM patients, CRP is a non-specific acute-phase reactant influenced by various inflammatory conditions. The observed high values (up to 175 mg/L) in some patients, despite our exclusion criteria, may reflect subclinical inflammation or the extensive systemic impact of the malignancy itself. CRP is a supportive clinical indicator reflecting the systemic inflammatory environment and disease severity in MM. Its utility in clinical practice should be considered as part of a comprehensive assessment of the patient's overall inflammatory status during the initial evaluation, rather than as an independent triage parameter.

### Limitations

The retrospective and cross-sectional design of our study limits its capacity to provide a diagnostic or screening context. Our current findings should be interpreted not as a screening test, but as clinical indicators that reflect the disease severity and systemic impact of MM at the time of presentation. We believe that recognizing these deviations in routine parameters can provide valuable clinical insights into the severity of the disease process.

The retrospective design of the study resulted in missing data for some parameters. This situation led to varying sample sizes across different analyses depending on the availability of the data records.

Furthermore, our study population did not include individuals with pre-malignant conditions such as Monoclonal Gammopathy of Undetermined Significance (MGUS) or Smoldering Multiple Myeloma (SMM). Therefore, our findings should be interpreted as indicators of disease severity in symptomatic MM rather than markers for the early screening of asymptomatic precursor states.

Another limitation of our study is the selection of the control group. In our study, MM patients were compared with healthy individuals; however, in real-world clinical practice, the diagnostic challenge often involves distinguishing MM from other conditions such as iron deficiency anemia, anemia of chronic disease, chronic kidney disease, or other inflammatory disorders. Comparing MM patients with healthy controls rather than a patient-based control group limits the generalizability of our findings to routine clinical triage where these comorbidities are frequently encountered.

### Conclusion

Most malignancy patients present to healthcare institutions with low-risk and non-specific symptoms. Careful evaluation of MM patients' non-specific laboratory findings, which correlate with markers of tumor load such as M-protein and  $\beta$ -2M, is important for assessing the systemic involvement of the disease at the time of presentation. In conclusion, these routine parameters serve as valuable clinical indicators reflecting the overall disease severity and systemic impact of MM.

### Disclosures

**Ethics Committee Approval:** The study was approved by the Kayseri City Training and Research Hospital Ethics Committee (no: 376, date: 25/03/2025).

**Informed Consent:** Written informed consent was obtained.

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