Predictive Immun Markers for Disease Progression in Multiple Myeloma (MM) and Monoclonal Gammapathy of Undetermined Significance (MGUS)

Sahver Isgor I. et al: Progression Predictive Immune Markers in MM and MGUS

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Abstract

Objectives: Multiple Myeloma (MM) and Monoclonal Gammopathy of Undetermined Significance (MGUS), the precursor condition of MM, are plasma cell neoplasms. The evolution of the treatment for MM in recent years has dramatically improved the outcome for these patients. Currently, multidisciplinary studies are conducted to elucidate the etiopathogenesis of the disease and develop specific treatment agents and prognostic markers. To investigate the relationship between immunoeexpression of CD138, Pan-Ras, CCL-3, DKK-1, MUM1, and disease progression in cases with MM and MGUS.

Materials and Methods: IHC staining for CD138, Pan-Ras, CCL-3, DKK-1, and MUM1 was performed on bone marrow biopsy samples from 94 MM and 20 MGUS patients diagnosed between 2011 and 2018. Immunohistochemistry data were examined semi-quantitatively, and the association between IHC, clinical, and biochemical markers utilized for MM and MGUS patient staging were analyzed.

Results: Pan-Ras, DKK-1 and MUM-1 staining were significantly higher in MM cases compared to MGUS cases (p = 0.005, 0.001, and 0.001, respectively). The mean CCL-3 expression in patients with MGUS was 23.15 %, while it was 18.68 % in cases with MM (p=0.413). CCL-3 expression was significantly higher in high-risk MGUS cases compared to other groups, according to the Mayo Clinic risk stratification (MCRS) model for MGUS. According to the ISS and R-ISS, CD138 expression was higher in stage 2 and stage 3 patients than in stage 1 patients.

Conclusion: Differences in Pan-Ras, MUM-1, DKK-1, and CCL-3 expression between MM and MGUS suggest that these molecules may play a role in the progression of MGUS-MM. CCL-3, an immunohistochemical marker, may be predictive in MGUS progression, while CD138 is associated with more advanced stages of MM.

Keywords: MM, MGUS, DKK-1, Pan-Ras, CCL-3, MUM-1

Introduction

Multiple myeloma (MM) is a plasma cell neoplasm defined by the proliferation of clonal plasma cells in the bone marrow and the release of a monoclonal protein in serum and/or urine in the majority of cases. MM accounts for 10-15% of hematological malignancies and 1% of all malignancies, with a median age at diagnosis of 69 years [1, 2]. In recent years, the advancement of MM treatment has greatly improved the outcomes for these patients [3,4,5]. Monoclonal gammopathy of unknown significance (MGUS) is an asymptomatic precursor plasma cell neoplasm that progresses to MM in roughly 1% of cases per year. The etiopathogenesis for progression from MGUS to MM has not been elucidated yet [2,6,7,8]. Many preclinical and clinical investigations have been conducted to investigate the cytogenetic pathogenesis of MGUS and MM. The hematopoietic niche environment is critical
in the genesis and progression of MGUS and MM. A tremendous effort has recently been made to produce niche-oriented therapeutic agents [3, 4]. Treatment regimens for lytic lesions in the bone, which are regarded as one of the most serious consequences of the disease, have gained ground in the research pool [5].

To estimate the prognosis of MM, many risk scoring systems have been designed. The International Staging System is the most frequently approved and used of them (ISS). Serum 2-microglobulin and serum albumin levels are used in this staging system [9].

With a focus on molecular pathogenesis and the prognostic utility of molecular changes, the International Staging System (ISS) was recently revised by including cytogenetic high-risk-related mutations and serum lactate dehydrogenase levels, and a revised International Staging System (R-ISS) was created [10,11,12]. The use of these staging systems, however, varies by institution.

There are efforts to stratify MGUS cases according to the risk of progression. Mayo Clinic’s risk classification model for MGUS is the most recognized of these efforts, and it is also referenced in the International Myeloma Working Group (IMWG) 2010 manual. After 20 years of follow-up, the Mayo Clinic model advises that cases be classified as low-risk, low-medium risk, medium-high risk, or high-risk [1, 2, 6, 7].

In MM and MGUS, myeloma cells and atypical plasma cells express Syndecan-1, also known as CD138, an integral membrane protein that allows the cells to communicate with the extracellular matrix [13]. CD138 has been linked to myeloma cell adhesion and communication [13,14,15,16].

Chemokine ligand 3 (CCL-3) / Macrophage Inflammatory Protein (MIP-1) is a chemokine produced by myeloma cells that are involved in the niche stage of MM and MGUS tumor pathogenesis. CCL-3 receptors were found on bone marrow-derived stem cells (BMSC), osteoclasts, and osteoblasts. CCL-3 has also been linked to disease-related bone damage [17, 18]. Another function of CCL3, which is significant in the pathogenesis of MM, is its role in myeloma cell survival in the bone marrow niche [3, 19, 20].

MUM-1 / IRF-4 (Multiple Myeloma Oncogene) is a gene that belongs to the interferon regulator family and is associated with the different stages of plasma cell development. It is involved in the development of MM and its precursor forms, the differentiation of T-helper (Th17) cells, which is crucial in the tumor microenvironment, and cytokine release. Several studies [21,22,23] have found that immunoexpression of MUM-1 has increased in the advanced stages of MM.

Dickkopf gene-1 is one of the cytokines that play a key role in the relationship between tumor and microenvironment (DKK-1). DKK-1 inhibits osteoblastogenesis via antagonizing the Wingless Integrated Cell Signaling Pathway (WNT-1). Nevertheless, in MM, a similar mechanism stops BMSCs from maturing into mature osteoblasts. The increasing number of bone complications caused by DKK-1 mediated events in advanced stages of MM suggests that the DKK-1 immunohistochemistry marker can be used to determine prognosis [18, 24,25,26].

Although K-RAS, N-RAS, and H-RAS mutations are mostly associated with epithelial neoplasms, they are also detected at a frequency of 9-30% in MM. Although this rate has been detected in MGUS and other MM precursor diseases, it is noteworthy that the frequency of mutations is higher in MM compared to MGUS. Patients with MM have a poorer prognosis if there is a mutation in RAS genes. Several studies have reported mutations of RAS subtypes at varying frequencies in MM pathogenesis [27,28,29,30].

The aim of the study was to evaluate the impact of a set of immunohistochemical markers on disease progression in bone marrow biopsies of MM and MGUS patients.

Materials and Methods

Patients
The study was designed at Marmara University School of Medicine's Department of Pathology. At the beginning of the study, bone marrow biopsies were used to make the initial diagnosis, and the pathology department at Marmara University School of Medicine Pendik Research Hospital investigated the data of 443 patients diagnosed with "Multiple Myeloma or Monoclonal Gammapathy of Undetermined Significance" between January 2011 and March 2018. Biopsies for remission and/or recurrence evaluation were not included. The study comprised bone marrow specimens from 20 patients with MGUS and 94 cases with MM who had records of disease stages and cytogenetic analysis results. Clinical data were gathered from patient records in the same center's hematology department. The hematologist informed us about the ISS, R-ISS, and MCRS system data for the patients, which are disease staging systems. The amounts of serum 2 microglobulin and albumin were used to assess ISS. Serum lactate dehydrogenase levels and cytogenetic data were added to the ISS to assess the R-ISS. Non-IgG isotype (IgA and IgM), M protein levels, and serum-free light chain ratio were all determined in MGUS patients using the MCRS system. Patients who did not have sufficient clinical data or tissue for immunohistochemistry analyses were excluded. The Ethics Committee of Marmara University approved the study (09.2018.277).

**Immunohistochemical analysis**

Bone marrow biopsies were preserved in formalin-fixed paraffin-embedded (FFPE) blocks after three hours of decalcification in ethylenediaminetetraacetic acid (EDTA) solution (concentration of 10%). Four μm thick sections were mounted on positively charged slides. Staining of CCL-3 (PAI-38160, polyclonal, rabbit, Thermo Fischer, 1:50), MUM-1 (DO-7, monoclonal, Mouse, Dako, ready-to-use), DKK-1 (SAB1404944, monoclonal, mouse, Sigma-Aldrich, 1:100), Pan-Ras (Ras10, monoclonal, mouse, Thermo Fischer, 1:100) markers was performed using an automated immunohistochemistry device (Ventana medical systems inc, BenchMark Ultra XT automated stainer, Ventana Medical System, Inc., Tucson, AZ, USACCL-3). CD138 (MI15, monoclonal, mouse, Cellmark, 1:100), Kappa light chain (L1C1, monoclonal, mouse, Thermo Scientific-Lab Vision, 1:200), and lambda light chain (HP6054, monoclonal, mouse, Genemed, 1:50) immunohistochemistry were performed by re-evaluating the first diagnosis slides of the cases using the same techniques. Cytoplasmic staining with Pan-Ras, CCL-3, and DKK-1; membranous staining with CD138, and nuclear staining with MUM-1 were considered positive. Results were noted by two blind researchers who specialized in pathology. While calculating staining percentages of Pan-Ras, CCL-3, DKK-1, and MUM-1 markers, it was performed by scanning all slides, then CD138 (+) neoplastic cells were taken into count and a total number of hematopoietic cells were not counted. A ratio of any given marker represents the positive staining cell percentage of the total number of neoplastic plasma cells.

**Statistical analysis**

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used to evaluate the data. Shapiro-Wilk test and graphical analysis were used to control whether the quantitative data were normally distributed. The student’s t-test was used to compare normally distributed quantitative variables and the Mann-Whitney U test was used to compare non-normally distributed quantitative variables between two groups. The Kruskal Wallis test was used to compare the quantitative variables that were not normally distributed between three or more groups, and the Bonferroni-Dunn test was used for pairwise comparison. Pearson Chi-Square test and Fisher's Exact test were used to comparing qualitative data. Statistical significance was accepted as p <0.05.

**RESULTS**

**Demographic characteristics**
The mean age of all cases was 65 (27-92) years and the M/F ratio was 1.08.

**Staging and results of clinical risk scores**

The patients were classified at the time of diagnosis according to the ISS: 44 patients (46.8%) had stage I, 20 (21.2%) had stage II, and 30 (32%) had stage III. The patients were reclassified at diagnosis according to the R-ISS: 17 patients (18%) had stage I, 56 (59.5%) had stage II, and 21 (22.5%) had stage III are shown in Table 1. When the patients were classified according to the ISS, 44 patients (46.8%) were in stage I, 20 (21.2%) were in stage II, and 30 (32%) were in stage III. When R-ISS was used for classification, 17 patients (18%) were at stage I, 56 patients (59.5%) were at stage II, and 21 (22.5%) were at stage III (Table 1).

Patients with MGUS were classified according to Mayo Clinic Risk Classification Model. 4 patients (20%) were in a low-risk group, 7 (35%) were in low to the intermediate-risk group, 5 (25%) were in high to the intermediate-risk group, and 4 (20%) were at the high-risk groups (Table 2).

**Immunohistochemical findings**

Results of immunohistochemical examinations are shown in Table 3. According to these data, Pan-Ras staining rates were higher in cases with MM than those with MGUS (p = 0.005). Staining patterns of Pan-Ras are shown in Figure 1. There was no statistically significant difference between the cases with MM and MGUS in terms of CCL-3 staining rates (p> 0.05). DKK-1 and MUM-1 expression levels of MM were significantly higher than of MGUS (p = 0.001 and p = 0.001, respectively). The staining patterns of MUM-1 are shown in Figures 1 and Figure 2.

**Analysis of the relationship between immunohistochemical findings and ISS and R-ISS in cases with MM**

CD138 staining rates were higher in advanced ISS stages (p = 0.002) (Table 4). This result suggests that there are more atypical plasma cells in the advanced ISS groups. After pairwise comparisons, which were made to determine the group causing the significant difference, the staining rate of stage III cases was found to be higher than stage I cases (p = 0.003). When R-ISS classification was taken into account, advanced stages also showed higher rates of CD138 staining (p = 0.043) (Table 5). The pairwise comparisons showed that the staining rates of stage III cases were higher than those with stage I and II (p = 0.037). According to the R-ISS stages Pan-Ras, CCL-3, and MUM-1 showed higher expression rates at advanced stages, but these differences were not statistically significant.

Comparison of IHC findings according to ISS (I-II-III) and R-ISS (I-II-III) stages are shown in Table 4 and Table 5.

**Analysis of the Relationship Between Immunohistochemical Findings and the Mayo Clinic Risk Stratification Model Groups in Cases with MGUS**

The correlation between the results of immunohistochemical stainings and the risk groups is shown in Table 6. "No risk / Low risk / Medium risk" groups were evaluated together and the "High risk" group was evaluated separately. CCL-3 staining rates of cases that were classified as “high-risk” according to MCRS criteria were found to be significantly higher than other groups (p = 0.018). Although statistically insignificant immunoeexpression of all markers was increased in the “high-risk” group compared to the "no risk / low risk/medium risk" groups. There was no correlation between expression rates of CCL-3 and other markers.

**Discussion**

MM and MGUS are plasma cell neoplasms and the etiopathogenesis of these diseases has not been fully clarified. They especially affect people over 65 years of age, and despite the great
advance in treatment, MM remains a largely incurable disease. The mechanisms for disease progression have also not been elucidated [1, 2, 6, 7, 30]. Several studies have reported different rates of distribution according to ISS and R-ISS stages at the time of diagnosis in cases with MM [10, 11]. Palumbo et al. have reported that 38% of the participants were ISS stage 1, 38% were ISS stage 2, and 24% were ISS stage 3 [10]. Zepeda et al. have reported that 30.3% of the patients were R-ISS stage 1, 46.5% were R-ISS stage 2, and 23.2% were R-ISS stage 3 [11]. In our study, 46.8% of the patients were ISS stage 1, 21.3% were ISS stage 2, 31.9% were ISS stage 3, while 18.1% were R-ISS stage 1, 59.6% were R-ISS stage 2, and 22.3% were R-ISS stage 3.

When we categorized MGUS cases using the Mayo Clinic risk stratification model criteria, we found that four (20%) were in the "No risk factor" group, seven (35%), were in the "low-moderate risk" group, five (25%) in the "medium-high risk" group, and four (20%) in the "high risk" group. Rajkumar et al. reported that the majority of cases in a large series of patients were classed as "medium risk" at the time of diagnosis, which is similar to our cohort [6, 8].

Numerous findings in the literature focus on the immunoexpression rate and staining pattern of CD138, as well as its association with disease prognosis in MM and its precursors [14, 16, 31]. Kim et al. studied CD138 levels using serum immunoelectrophoresis and suggested a possible link between CD138 levels and disease stage [32]. In our study, we discovered a statistically significant difference between CD138 staining rates and ISS and R-ISS stages in MM patients, that was consistent with previous research [14, 15, 16]. Kawano et al. and Foster et al. found an increase in CD138 expression using flow cytometry and immunohistochemistry, and they believed that this increase was related to the disease's poor prognosis [31, 33]. When we evaluated CD138 staining in MGUS cases using MCRS criteria, we found no significant difference. This conclusion could be due to the narrow distribution range of the plasma cell ratio (3-9%) in MGUS disease, as well as the small number of cases included in our investigation.

Many similar mutations have been found in cytogenetic anomalies in MM and MGUS. One of these mutations is in the RAS gene, which has been linked to the development of MM in patients with MGUS [28, 30]. RAS has been linked to the development of various cancers, including hematological malignancies [28, 29, 30]. Zangari et al. have reported that inhibition of Pan-Ras has prevented the development and progression of MM in rats [34]. Our study has shown that the rate of Pan-Ras immunoexpression in plasmacytic cells was higher in MM patients than in MGUS patients (p = 0.005). This finding gives support to Pan-Ras significance in disease progression. This observation is further supported by the literature on the role of the RAS gene in MM development from MGUS [7, 27]. All of these findings suggest that the Pan-Ras immune marker can be utilized to evaluate patients with MGUS and early-stage MM, and that therapeutic agent can be developed accordingly. Another possible supporting evidence of the role of Pan-Ras in plasma cell neoplasm progression is its higher expression levels in advanced risk groups. When our cohort's MGUS patients were classified using the MCRS system, the Pan-Ras immunoexpression ratio was higher in the high-risk group, but this difference was not statistically significant (Table 6). We found no correlation between Pan-Ras expression and ISS stages. However, when the results are evaluated using R-ISS criteria, which involve cytogenetic analysis in addition to ISS criteria, it is clear that the expression of Pan-Ras has increased in higher R-ISS stages (means 36.8 percent- 37.15 percent and 46.50 percent in R-ISS stage I, II and III, respectively). When all of these data were evaluated together, the presence of a statistically significant difference between cases with MGUS and MM, as well as a difference between stages in the R-ISS system in terms of increased expression, indicated consistency with the pathogenesis of RAS mutations in
MGUS-MM progression [7,27]. These findings suggest that the Pan-Ras immune marker could be utilized to follow the progression of MGUS-MM and predict the progression of MM. MIP-1α (Macrophage inflammatory protein-1 alpha / CCL-3) is a chemotactic cytokine released by macrophages. CCL-3 also stimulates the synthesis of molecules that promote the proliferation of MM cells in the bone marrow niche, such as receptor activator of nuclear factor-kappa-B ligand (RANKL), interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), and tumor necrosis factor (TNF). CCL-3 also plays an active role in the early stages of neoplasm, as in the neoplastic cell adhesion to the microenvironment [26]. This theory is supported by a higher rate of immunoexpression in the high-risk group compared to other MCRS groups. CCL-3 may also have a part in the development of lytic bone lesions in MM pathogenesis by inhibiting osteoblastic cells and activating osteoclastic cells [25, 35]. Politou et al. explain the mechanism of action of CCL-3 with the change in the osteoblastic/osteoclastic activity balance in the bone, primarily and more predominantly by reducing osteoblastic activity [20]. Palma et al. found that elevated levels of CCL-3 and DKK-1 are associated with lytic bone lesions in MM, MGUS, and Smoldering Multiple Myeloma (SMM) patients [25]. Ng et al. found unexpectedly high levels of CCL-3 in the serum of MGUS patients in their study. Although lytic bone lesions are not expected in MGUS, they did discover impaired bone formation in MGUS patients. They explained this discovery to the advanced radiological examination method they used in their study. As a result, they were able to identify clinically unexpected lytic bone lesions at the microstructural level in MGUS cases [24]. All these data may suggest that CCL-3 has different roles in different stages of the pathogenesis of plasmacytic neoplasms. In addition to predicting an advanced stage of MGUS, we can also suggest that high CCL-3 levels may portend possible progression of bone lesions in plasma cell neoplasms and treatment regimens can be developed accordingly.

Dickkopf-1 (DKK-1) is a cytokine expressed by myeloma cells and another molecule involved in the development of bone lesions in MM. Because there is no definite treatment for bone complications in MM, research on this marker has grown in popularity [5, 18, 26]. In our study, we found that DKK-1 expression was higher in MM patients than in MGUS patients. Palma et al. evaluated DKK-1 levels in bone marrow aspirations in patients with SMM and found that levels were higher in patients with progressive SMM. They hypothesized that DKK-1 could be a valuable marker for disease progression [25]. Even though there is no statistically significant difference between the high-risk and other risk groups of the MCRS system in our study, increased DKK-1 expression in the high-risk group shows that it may be a useful marker in assessing the progression risk of MGUS cases. Ng et al. found similar results for DKK-1 and CCL-3. They have explained this situation with bone lesions that are detectable at the microstructural level [24]. However, when we reviewed the current literature, we did not find any data that compared comparing DKK-1 levels in the progression of MGUS-SMM-MM.

MUM-1 plays critical roles in myeloma pathogenesis, including cell cycle control, energy metabolism, and cell death [21, 22]. Shaffer et al. found that when MUM-1 expression is inhibited, myeloma cells die suddenly [23]. In our study, we discovered that MUM-1 immunoexpression was substantially higher in cases of MM than in patients of MGUS. Hein tile et al. are the first to show that increased MUM-1 expression, as evaluated by polymerase chain reaction (PCR), is related to a worse prognosis in patients with MM [21]. According to the same study, the levels of MUM-1 expression detected by immunohistochemistry and PCR were not correlated, and more research on this topic was needed [21]. Although MUM-1 immunoexpression was not significantly correlated with ISS and R-ISS stages in our study, we observed that the expression rate increased in parallel with ISS and R-ISS staging (staining ratios for ISS I-II-III were 36.5%-41.2% -47.13%,
respectively) (staining ratios for R-ISS I -II-III were 32.24% -39.61% -51.43% respectively). Although not statistically significant, we found that MUM-1 expression was higher in the high-risk group (42%) compared to other groups when cases with MGUS were evaluated according to MCRS risk groups (8%). Based on these previous findings and our findings, we believe MUM-1 can be a reliable marker for assessing MGUS-MM progression and possibly predicting aggressive behavior in MM cases.

Based on the findings of the study, we propose that immunohistochemical evaluation of plasmacytic neoplasms can provide predictable information regarding the risk of development of MGUS to MM as well as the biological behavior of cases with MM. Immunohistochemical analysis has an advantage over molecular approaches in that it is a low-cost, quick method that may be performed in bone marrow biopsies at the time of diagnosis. In general, MGUS-SMM-MM progression and predicting MM aggressive behaviors are complicated, multistep, and multifactorial facts. Although CD138, Pan-Ras, DKK-1, MUM-1, and CCL-3 stainings seem to provide essential information in predicting those progressions, we believe that larger studies with additional various markers on this subject should be performed.

**Study Limitations**

This study has some limitations. The number of MGUS patients in the study is limited. Another limitation is that only the cases with cytogenetic analysis were included in the study. Statistically insignificant results we had while evaluating the effectiveness of markers may be due to a limited number of cases.

**Conclusion**

In our examination of MM and MGUS patients, we discovered that Pan-Ras, DKK-1, and MUM-1 were expressed at higher rates by neoplastic cells in MM cases than in MGUS cases. This finding shows that the aforementioned molecules may play essential roles in the course of MGUS-MM. Immunotherapeutic agents for these markers may be considered for treatment options. Because the CD138 expression rate represents the number of plasma cells in bone marrow, it is much higher in advanced ISS and R-ISS stages. This result demonstrates that the percentage of CD138 expression could be associated with a more advanced stage by showing the neoplastic plasma cell burden in the bone marrow and can be used as a predictive marker for aggressive behavior in MM patients, even though it is not used in the ISS and R-ISS systems.

The increased expression of CCL-3 in MGUS cases compared to MM, as well as higher expression of CCL-3 in the high-risk group of MGUS compared to the other groups according to MCRS, could be crucial in early MM oncogenesis.

Our research focuses on immunohistochemistry markers that are significant in the pathogenesis of plasma cell neoplasms, as well as the predictive utility of those markers in plasma cell neoplasm progression. Further studies are warranted to better assess the role of immunohistochemical biomarkers, such as markers indicating tumor proliferative capacity, in MGUS progression and MM prognosis.

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**Conflicts of interest/Competing interests:**
Irem Sahver Isgor declares that he has no conflict of interest.
Huseyin Kemal Turkoz declares that he has no conflict of interest.

**Availability of data and material:** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Code availability:** Not applicable

**Authors’ contributions:** All authors contributed the study conception and design. Material preparation, data collection and analysis were performed by Irem Sahver Isgor. The first draft
of the manuscript was written by Irem Sahver Isgor and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Ethics approval**

The study protocols were approved by the Ethical Board of Marmara University Medical School, Istanbul, Turkey (Protocol number: 09.2018.277)

**Consent to participate:**

Conseptualization: Irem Sahver Isgor, Huseyin Kemal Turkoz
Methodology: Irem Sahver Isgor
Formal analysis and investigation: Irem Sahver Isgor
Data collection-histopathological and immunohistochemical: Irem Sahver Isgor
Data collection-clinical: Irem Sahver Isgor, Tayfur Toptas
Funding acquisition: Irem Sahver Isgor, Huseyin Kemal Turkoz

**References**


Figure 1 Expression of immunohistochemical markers in MM (A) MUM-1 expression in atypical plasma cells, 90%, 400x (B) Pan-Ras expression in atypical plasma cells, 30%, 400x (C) DKK-1 expression in atypical plasma cells, 95%, 200x (D) CCL-3 expression in atypical plasma cells, 80%, 400x

Figure 2 Expression of immunohistochemical markers in MGUS (A) Hematoxylin-Eosin staining, 40x (B) CD138 expression in atypical plasma cells, 6%, 40x (C) MUM-1 expression in atypical plasma cells, 80%, 40x (D) Pan-Ras expression in atypical plasma cells, 0%, 40x
Table 1. The ISS and R-ISS distribution in Multiple Myeloma cases

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Table 2. The Mayo Clinic Risk Stratification Model distribution in MGUS cases

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Table 3. Comparison of the immunohistochemical results of MM and MGUS cases
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<th>Multiple Myeloma (n=94)</th>
<th>MGUS (n=20)</th>
<th>p</th>
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</thead>
</table>
| CD138 (%) | Min-Max
(Median) | 3-100 (60) | 15-100 (62.5) | 3-9 (6.8) | **0.001** |
|         | Mean±Sd       | 50.48±30.06            | 59.89±24.22 | 6.22±1.73 |         |
| Pan-Ras (%) | Min-Max
(Median) | 0-100 (40) | 1-100 (40) | 0-60 (10) | **0.005** |
|         | Mean±Sd       | 36.54±29.20            | 39.97±29.80 | 20.40±19.79 |         |
| CCL-3 (%) | Min-Max
(Median) | 0-80 (10) | 1-80 (10) | 0-80 (20) | **0.413** |
|         | Mean±Sd       | 19.46±19.00            | 18.68±18.21 | 23.15±22.52 |         |
| DKK-1 (%) | Min-Max
(Median) | 0-95 (9.5) | 0-95 (10) | 0-20 (1.5) | **0.001** |
|         | Mean±Sd       | 15.26±18.36            | 17.79±19.17 | 3.40±5.20 |         |
| MUM-1 (%) | Min-Max
(Median) | 0-90 (30) | 1-90 (40) | 0-80 (2) | **0.001** |
|         | Mean±Sd       | 36.49±29.09            | 40.91±28.33 | 15.70±23.50 |         |

*Mann Whitney U Test, *p<0.05

Table 4. Comparison of the ISS stages and immunohistochemical results
Table 5. Comparison of IHC findings according to R-ISS stages

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<td>15-95 (70)</td>
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<td>Pan-Ras (%)</td>
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<td></td>
<td>1-60 (10)</td>
<td>1-80 (20)</td>
</tr>
<tr>
<td></td>
<td>Mean±Sd</td>
<td>11.12±13.75</td>
</tr>
<tr>
<td>DKK-1 (%)</td>
<td>Min-Max (Median)</td>
<td>Min-Max (Median)</td>
</tr>
<tr>
<td></td>
<td>2-50 (10)</td>
<td>0-95 (10)</td>
</tr>
<tr>
<td></td>
<td>Mean±Sd</td>
<td>17.24±16.79</td>
</tr>
<tr>
<td>MUM-1 (%)</td>
<td>Min-Max (Median)</td>
<td>1-80 (30)</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Mean±Sd</td>
<td>32.24±27.34</td>
<td>39.61±27.94</td>
</tr>
</tbody>
</table>

*Kruskal Wallis Test  
*p<0.05

### Table 6. Comparison of IHC findings of patients in high-risk groups with patients in other groups according to the Mayo Clinic risk stratification model for MGUS

<table>
<thead>
<tr>
<th>MGUS (n=20)</th>
<th>The Mayo Clinic risk stratification model</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No risk/ Low risk/ Low to intermediate risk groups (n=16)</td>
<td>High risk (n=4)</td>
</tr>
<tr>
<td>CD138 (%)</td>
<td>Min-Max (Median)</td>
<td>3-9 (6.8)</td>
</tr>
<tr>
<td></td>
<td>Mean±Sd</td>
<td>6.24±1.85</td>
</tr>
<tr>
<td>Pan-Ras (%)</td>
<td>Min-Max (Median)</td>
<td>0-60 (10)</td>
</tr>
<tr>
<td></td>
<td>Mean±Sd</td>
<td>17.25±18.08</td>
</tr>
<tr>
<td>CCL-3 (%)</td>
<td>Min-Max (Median)</td>
<td>0-70 (10)</td>
</tr>
<tr>
<td></td>
<td>Mean±Sd</td>
<td>17.06±17.12</td>
</tr>
<tr>
<td></td>
<td>Min-Max (Median)</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>------------------</td>
<td>-------</td>
</tr>
<tr>
<td>DKK-1 (%)</td>
<td>0-20 (1)</td>
<td>0-10 (6)</td>
</tr>
<tr>
<td>Mean±Sd</td>
<td>2.88±5.21</td>
<td>5.50±5.26</td>
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<tr>
<td>MUM-1 (%)</td>
<td>0-60 (2)</td>
<td>1-80 (45)</td>
</tr>
<tr>
<td>Mean±Sd</td>
<td>8.94±15.59</td>
<td>42.75±32.61</td>
</tr>
</tbody>
</table>

*aMann Whitney U Test  *p<0.05