Significance of Elevated NGAL, TIM-1, VCAM-1, and Activin A in Patients with Newly Diagnosed Multiple Myeloma: A Postprint Study

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Date: 2024-10-30T00:00:00+00:00

Abstract

Background Multiple myeloma (MM) is a malignant plasma cell disorder. Nearly half of patients have MM-related kidney injury (KI) at diagnosis, which is associated with increased patient mortality. Early identification and treatment of KI may reverse renal function and improve patient survival. Neutrophil gelatinase-associated lipocalin (NGAL), T cell immunoglobulin mucin receptor 1 (TIM-1), vascular cell adhesion molecule-1 (VCAM-1), and activin A are associated with pathological processes such as cell proliferation, invasion, and KI. These indicators show promise as relevant markers for early diagnosis of MM-related KI, but current research is limited and further exploration is needed. Objective To investigate the significance of novel biomarkers NGAL, TIM-1, VCAM-1, and activin A in disease evolution, staging, and prognosis of newly diagnosed MM (NDMM) complicated with KI. Methods We selected 70 hospitalized patients with NDMM admitted to the Hematology Ward of Shijingshan Campus, Beijing Chaoyang Hospital, Capital Medical University between January 2017 and February 2022, including 62 cases of symptomatic MM and 8 cases of smoldering MM (SMM). Additionally, 12 patients with monoclonal gammopathy of undetermined significance (MGUS), 7 patients with monoclonal gammopathy of renal significance (MGRS), and 20 healthy control (HC) subjects were also enrolled in this study. Clinical data of all subjects were collected, and bone marrow aspiration and biopsy, M protein examination, and imaging examination results were collected for MGUS, MGRS, and NDMM patients. Enzyme-linked immunosorbent assay (ELISA) was used to detect urinary NGAL (uNGAL), urinary TIM-1 (uTIM-1), serum TIM-1 (sTIM-1), urinary VCAM-1 (uVCAM-1), and urinary activin A concentrations. According to disease status, subjects were divided into MGUS group, MGRS group, NDMM group, and HC group; according to disease status, patients were divided into MGUS group, MGRS group, NDMM

group, and HC group; according to KI status, patients were divided into MGUS group, MGRS group, KI group, and non-kidney injury (NKI) group. The KI group was further divided into pre-treatment MM subgroup, post-treatment achieving PR or better (>PR) subgroup, and relapsed MM subgroup based on dynamic disease treatment status. Spearman correlation analysis was used for correlation analysis of each indicator. Receiver operating characteristic (ROC) curves were plotted to analyze the diagnostic value of each detection factor for NDMM complicated with KI, and optimal cutoff values and other indicators were calculated. Based on the optimal cutoff values of each detection factor, patients were divided into above-optimal-cutoff group and below-optimal-cutoff group. Kaplan-Meier method was used to analyze the overall survival (OS) status of each detection factor in the above-optimal-cutoff and below-optimal-cutoff groups. Cox proportional hazards regression analysis was used to analyze influencing factors for all-cause mortality in NDMM patients with KI. Results Levels of uNGAL, uTIM-1, uVCAM-1, sTIM-1, and urinary activin A in the NDMM group were higher than those in the MGUS group and HC group ($P<0.05$). Levels in the KI group were higher than those in the MGUS group, MGRS group, and NKI group ($P<0.05$). Levels in the pre-treatment MM subgroup were higher than those in the >PR subgroup ($P<0.05$). uNGAL, uTIM-1, uVCAM-1, sTIM-1, and urinary activin A were positively correlated with creatinine, $ $2-microglobulin, and R-ISS stage, and negatively correlated with estimated glomerular filtration rate (eGFR) ($P<0.05$). uNGAL, uTIM-1, sTIM-1, and urinary activin A were positively correlated with 24-hour urinary light chain level ($P<0.05$). uNGAL and uTIM-1 were positively correlated with clonal plasma cell percentage ($P<0.05$). Based on ROC curves, the optimal cutoff values for diagnosing MM-related KI were 14.774 ng/mL for uNGAL, 1.978 ng/mL for uTIM-1, 144.400ng/mL for uVCAM-1, and 33.730 pg/mL for urinary activin A. Kaplan-Meier analysis showed that patients with values above the optimal cutoff values for uNGAL, uTIM-1, sTIM-1, uVCAM-1, and urinary activin A had worse OS ($P<0.05$). Multivariate Cox proportional hazards regression analysis showed that uNGAL and R-ISS stage were influencing factors for all-cause mortality in NDMM patients with KI ($P<0.05$). Conclusion uNGAL, uTIM-1, uVCAM-1, and urinary activin A may be associated with MM disease progression and KI, and are early markers of renal tubular injury, which is beneficial for early diagnosis and treatment of MM patients with KI. They are also correlated with tumor burden indicators such as R-ISS stage, 24-hour urinary light chain, and clonal plasma cell percentage, as well as overall survival in MM patients, suggesting that these factors not only serve as effective supplements to novel KI biomarkers in clinical practice beyond serum creatinine, but may also have potential as new anti-MM therapeutic targets in the future.

Full Text

Significance of Elevated NGAL, TIM-1, VCAM-1, and Activin A in Patients with Newly Diagnosed Multiple Myeloma

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Abstract

Background: Multiple myeloma (MM) is a malignant plasma cell disorder, with nearly half of patients presenting with MM-related kidney injury (KI) at diagnosis, which is associated with increased mortality. Early recognition and treatment of KI may reverse renal dysfunction and improve patient survival. Neutrophil gelatinase-associated lipocalin (NGAL), T-cell immunoglobulin mucin receptor 1 (TIM-1), vascular cell adhesion molecule-1 (VCAM-1), and Activin A are involved in pathological processes including cell proliferation, invasion, and KI. These biomarkers show promise for early diagnosis of MM-related KI, but current research remains limited and requires further investigation.

Objective: To investigate the significance of novel biomarkers NGAL, TIM-1, VCAM-1, and Activin A in disease evolution, staging, and prognosis of newly diagnosed MM (NDMM) patients with KI.

Methods: Seventy hospitalized NDMM patients admitted to the Department of Hematology at Shijingshan Campus, Beijing Chaoyang Hospital, Capital Medical University between January 2017 and February 2022 were enrolled, including 62 symptomatic MM and 8 smoldering MM (SMM) patients. Additionally, 12 patients with monoclonal gammopathy of undetermined significance (MGUS), 7 patients with monoclonal gammopathy of renal significance (MGRS), and 20 healthy controls (HC) were included. Clinical data were collected from all participants, and bone marrow biopsy, M protein examination, and imaging results were obtained for MGUS, MGRS, and NDMM patients. Urinary NGAL (uNGAL), urinary TIM-1 (uTIM-1), serum TIM-1 (sTIM-1), urinary VCAM-1 (uVCAM-1), and urinary Activin A concentrations were measured by enzyme-linked immunosorbent assay (ELISA). Patients were strati-

fied into MGUS, MGRS, NDMM, and HC groups based on disease status, and into MGUS, MGRS, KI, and non-renal injury (NKI) groups based on renal involvement. The KI group was further divided into pre-treatment MM, post-treatment >PR (partial response), and relapsed MM subgroups according to dynamic treatment status. Spearman correlation analysis was used to assess relationships between variables. Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic value of each biomarker for NDMM with KI, and optimal cutoff values were determined. Patients were classified into above-cutoff and below-cutoff groups based on these thresholds. Kaplan-Meier analysis was performed to evaluate overall survival (OS), and Cox proportional hazards regression was used to identify factors influencing all-cause mortality in NDMM patients with KI.

Results: The NDMM group exhibited significantly higher levels of uNGAL, uTIM-1, uVCAM-1, sTIM-1, and urinary Activin A compared to MGUS and HC groups ($P<0.05$). The MGRS group also showed elevated levels of these markers compared to the MGUS group ($P<0.05$). The KI group demonstrated higher levels than the NKI group ($P<0.05$). Both pre-treatment and relapsed MM subgroups had higher levels than the >PR subgroup ($P<0.05$). uNGAL, uTIM-1, uVCAM-1, sTIM-1, and urinary Activin A were positively correlated with creatinine, $ $2-microglobulin, and R-ISS stage, and negatively correlated with estimated glomerular filtration rate (eGFR) ($P<0.05$). uNGAL, uTIM-1, sTIM-1, and urinary Activin A were positively correlated with 24-hour urinary light chain levels ($P<0.05$). uNGAL and uTIM-1 were positively correlated with clonal plasma cell percentage ($P<0.05$). ROC analysis identified optimal cutoff values for diagnosing MM-related KI: uNGAL 14.774 ng/mL, uTIM-1 1.978 ng/mL, uVCAM-1 144.400 ng/mL, and urinary Activin A 33.730 pg/mL. Kaplan-Meier analysis revealed worse OS in patients above these cutoff values ($P<0.05$). Multivariate Cox analysis identified uNGAL and R-ISS stage as independent factors influencing all-cause mortality in NDMM patients with KI ($P<0.05$).

Conclusion: uNGAL, uTIM-1, uVCAM-1, and urinary Activin A are associated with MM disease progression and KI, serving as early markers of tubular injury that facilitate early diagnosis and treatment of MM patients with renal involvement. These biomarkers correlate with tumor burden indicators (R-ISS stage, 24-hour urinary light chain, clonal plasma cell percentage) and overall survival, suggesting they not only supplement creatinine as novel KI biomarkers but also represent potential therapeutic targets for MM.

Keywords: Multiple myeloma; Kidney injury; Prognosis; Neutrophil gelatinase-associated lipocalin; T-cell immunoglobulin and mucin domain 1; Vascular cell adhesion molecule 1; Activin A; Immunoglobulin light chain

1. Subjects and Methods

1.1 Study Subjects

We enrolled 70 newly diagnosed multiple myeloma (NDMM) patients admitted to the Department of Hematology at Shijingshan Campus, Beijing Chaoyang Hospital, Capital Medical University between January 2017 and February 2022, comprising 62 symptomatic MM and 8 smoldering MM (SMM) patients. Additionally, 12 patients with monoclonal gammopathy of undetermined significance (MGUS), 7 patients with monoclonal gammopathy of renal significance (MGRS), and 20 age- and sex-matched healthy controls (HC) were included. This study was conducted in accordance with the Declaration of Helsinki. All MGUS, MGRS, and MM patients were diagnosed at Beijing Chaoyang Hospital and provided informed consent approved by the hospital' s Ethics Committee and the medical school ethics committee (approval number: 2021-Ke-516-1).

Diagnostic Criteria: (1) Diagnosis, staging, and treatment response criteria for MGUS, MGRS, SMM, and NDMM patients followed the 2014 International Myeloma Working Group (IMWG) or 2022 National Comprehensive Cancer Network (NCCN) guidelines. (2) Kidney injury (KI) was defined according to new IMWG criteria as serum creatinine above the upper limit of normal or >2 mg/dL (176.8 mol/L) or estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m^2. eGFR was calculated using the simplified Modification of Diet in Renal Disease formula. (3) Revised International Staging System (R-ISS) criteria: R-ISS Stage I included ISS Stage I with standard-risk cytogenetics and normal lactate dehydrogenase (LDH); R-ISS Stage II included patients not meeting Stage I or III criteria; R-ISS Stage III included ISS Stage III with high-risk cytogenetics and elevated LDH. High-risk cytogenetic factors included del(17p) and/or t(4;14) and/or t(14;16), detected by fluorescence in situ hybridization (FISH).

Exclusion Criteria: (1) Patients receiving fewer than 2 cycles of chemotherapy; (2) Patients not achieving partial response (PR) or better; (3) Amyloidosis, plasma cell leukemia, or other malignancies. Initial induction therapy for MM patients primarily consisted of bortezomib-based regimens for 1-8 cycles (average 6-8 cycles). Patients achieving PR or better continued consolidation therapy or underwent autologous stem cell transplantation with 2-4 additional cycles of the original regimen. Patients not achieving PR after 4 induction cycles were switched to alternative regimens for optimal response. Most patients received lenalidomide or ixazomib maintenance therapy.

1.2 Patient Grouping

Patients were stratified into MGUS, MGRS, NDMM, and HC groups based on disease status, and into MGUS, MGRS, KI, and non-renal injury (NKI) groups based on renal involvement. The KI group was further divided into pre-treatment MM, post-treatment >PR (partial response), and relapsed MM subgroups according to dynamic treatment status.

1.3 Data Collection

Clinical data were collected from all subjects, including bone marrow biopsy and M protein examination results for MGUS, MGRS, and NDMM patients. Parameters recorded included age, sex, abnormal immunoglobulin heavy chain type, abnormal immunoglobulin light chain type, clonal plasma cell percentage, serum calcium, hemoglobin, $ 2 − $ $microglobulin, albumin, eGFR, creatinine, serum free light chain (sFLC) levels, serum involved − to − uninvolved FLC ratio (sFLC k/ $), 24-hour urinary protein, and 24-hour urinary light chain levels. Imaging studies (primarily X-ray and CT scans) were reviewed to determine the presence of bone lesions. Prognostic assessment of MM patients was based on FISH analysis of CD138+ clonal plasma cells enriched by magnetic bead sorting, using probes for D13S319, RB1, CKS1B(1q21)/CDKN2C(1p32), P53, IgH, IgH/FGFR3, IgH/MAF, IgH/MAFB, and IgH/CCND1.

1.4 Specimen Collection and Detection Methods

Urine and venous blood samples (5 mL each) were collected from all subjects, centrifuged at 2000 r/min for 10 minutes within 2 hours, and supernatants were stored at -80°C to avoid repeated freeze-thaw cycles. Urinary NGAL (uNGAL), urinary TIM-1 (uTIM-1), serum TIM-1 (sTIM-1), urinary VCAM-1 (uVCAM-1), and urinary Activin A were measured by ELISA using quantitative kits from R&D Systems (Minneapolis, MN, USA): ELISA Human Lipocalin-2/NGAL, ELISA Human Serum TIM-1/KIM-1/HAVCR, ELISA Human Urinary TIM-1/KIM-1/HAVCR, ELISA Human VCAM-1/CD106, and ELISA Human/Mouse/Rat Activin A. All assays were performed strictly according to manufacturer instructions, and the serum-to-urine TIM-1 concentration ratio was calculated.

1.5 Study Follow-up

NDMM, MGUS, and MGRS patients enrolled between January 2017 and February 2022 were followed until November 2022. Patients lost to follow-up for more than 6 months were contacted by mail or telephone, and death certificates were requested when applicable. Overall survival (OS), defined as time from diagnosis to death or last follow-up, was used for survival analysis.

1.6 Statistical Analysis

Statistical analysis and graphing were performed using SPSS 21.0 and GraphPad Prism 8.0. Normality was assessed by Kolmogorov-Smirnov test. Normally distributed variables were expressed as mean ± standard deviation and compared using independent samples t-test or one-way ANOVA. Non-normally distributed variables were expressed as median (interquartile range) and compared using Kruskal-Wallis H test with Mann-Whitney U test for pairwise comparisons, with results presented as violin plots. Correlation analysis was performed

using Spearman' s method. Categorical data were expressed as percentages and compared using ^{2} test with Bonferroni correction for multiple comparisons. ROC curves were constructed to calculate area under the curve (AUC), optimal cutoff values, sensitivity, and specificity. Patients were stratified into above-cutoff and below-cutoff groups based on these values. Kaplan-Meier analysis was used to assess OS and generate survival curves. Cox proportional hazards regression was used to identify factors influencing all-cause mortality in NDMM patients with KI. P<0.05 was considered statistically significant.

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2. Results

2.1 Comparison of Clinical Characteristics Among MGUS, MGRS, NDMM, and HC Groups

The NDMM group included 18, 33, and 19 patients with R-ISS Stage I, II, and III, respectively. FISH analysis revealed 21 patients with 0, 8 with 1, and 5 with 2 high-risk genetic abnormalities. Bone lesions were present in 0, 2, and 53 patients in the MGUS, MGRS, and NDMM groups, respectively. Significant differences were observed among the four groups in clonal plasma cell percentage, serum calcium, hemoglobin, $ 2 − $microglobulin, albumin, eGFR, creatinine, sFLC, sFLCk/ $, 24-hour urinary protein, and 24-hour urinary light chain levels (P<0.05). Pairwise comparisons showed that MGRS patients had higher creatinine and 24-hour urinary protein than HC (P<0.05). NDMM patients exhibited higher serum calcium, $ $2-microglobulin, and creatinine than HC (P<0.05). MGRS patients showed higher creatinine and 24-hour urinary protein but lower hemoglobin and eGFR compared to MGUS (P<0.05). NDMM patients had higher clonal plasma cell percentage, $ 2 − $microglobulin, sFLC, sFLCk/ $, 24-hour urinary protein, and 24-hour urinary light chain levels than both MGUS and MGRS groups (P<0.05), and lower albumin than MGUS (P<0.05) .

2.2 Comparison of Baseline Clinical Characteristics Among MGUS, MGRS, NKI, and KI Groups

The KI group included 2, 20, and 18 patients with R-ISS Stage I, II, and III, respectively, while the NKI group included 16, 12, and 1 patient with Stage I, II, and III. Significant differences were found among the four groups in clonal plasma cell percentage, serum calcium, hemoglobin, $ 2 − $microglobulin, albumin, eGFR, creatinine, sFLC, sFLCk/ $, 24-hour urinary protein, and 24-hour urinary light chain levels (P<0.05). Both KI and NKI groups showed higher clonal plasma cell percentages and lower hemoglobin levels compared to MGUS and MGRS (P<0.05). The KI group exhibited higher $ 2 − $microglobulin, creatinine, sFLC, sFLCk/ $, 24-hour urinary protein, and 24-hour urinary light chain levels than the NKI group (P<0.05), along with lower eGFR and hemoglobin compared to MGUS (P<0.05) .

2.3 Comparison of Clinical Characteristics Among Pre-treatment MM, >PR, and Relapsed MM Subgroups

Among the 20 patients in the pre-treatment MM subgroup (9 male, 11 female) and 11 in the relapsed MM subgroup (8 male, 3 female), significant differences were observed in clonal plasma cell percentage, serum calcium, hemoglobin, $ 2 − *microglobulin*, *albumin*, *eGFR*, *creatinine*, *sFLC*, *sFLCk*/ $, 24-hour urinary protein, and 24-hour urinary light chain levels (P<0.05). The >PR subgroup showed lower clonal plasma cell percentage, serum calcium, $ 2 − *microglobulin*, *creatinine*, *sFLC*, *sFLCk*/ $, 24-hour urinary protein, and 24-hour urinary light chain levels compared to both pre-treatment and relapsed MM subgroups (P<0.05), while demonstrating higher hemoglobin and eGFR than the pre-treatment subgroup (P<0.05) .

2.4 Comparison of Biomarker Levels Among HC, MGUS, MGRS, and NDMM Groups

Significant differences were observed among HC, MGUS, MGRS, and NDMM groups in uNGAL and uTIM-1 levels (P<0.05). Pairwise comparisons revealed that MGRS and NDMM groups had higher uNGAL levels than both HC and MGUS (P<0.05). MGRS and NDMM groups also showed higher uTIM-1 than HC (P<0.05), with NDMM higher than MGUS (P<0.05). Additionally, NDMM patients exhibited higher sTIM-1, TIM-1 ratio, uVCAM-1, and urinary Activin A levels compared to HC (P<0.05) .

2.5 Comparison of Biomarker Levels Among MGUS, MGRS, NKI, and KI Groups

Significant differences were found among MGUS, MGRS, NKI, and KI groups in uNGAL and uTIM-1 levels (P<0.05). MGRS and KI groups showed higher uNGAL and uTIM-1 than MGUS (P<0.05), and higher uNGAL than NKI (P<0.05). The KI group demonstrated higher uTIM-1 than NKI (P<0.05), along with elevated sTIM-1, TIM-1 ratio, uVCAM-1, and urinary Activin A compared to NKI (P<0.05) .

2.6 Comparison of Biomarker Levels Among HC, NKI, and KI Groups

Significant differences were observed among HC, NKI, and KI groups in uNGAL, uTIM-1, sTIM-1, TIM-1 ratio, uVCAM-1, and urinary Activin A levels (P<0.05). The KI group showed higher uNGAL, uTIM-1, uVCAM-1, and urinary Activin A than both NKI and HC (P<0.05), and higher sTIM-1 and TIM-1 ratio than NKI (P<0.05) [Figure 1: see original paper].

2.7 Comparison of Biomarker Levels Across MM Disease Stages

To further explore the relationship between biomarkers and disease evolution, NDMM patients were stratified into SMM, R-ISS Stage I, Stage II, and Stage

III groups. Significant differences were observed among HC, SMM, R-ISS Stage I, Stage II, and Stage III groups in uNGAL, uTIM-1, sTIM-1, uVCAM-1, and urinary Activin A levels (P<0.05). The SMM group showed higher sTIM-1, uVCAM-1, and urinary Activin A than HC (P<0.05). R-ISS Stage I patients exhibited higher uNGAL and uTIM-1 than HC (P<0.05), while R-ISS Stage III patients showed higher levels of all biomarkers compared to both Stage I and HC (P<0.05) [Figure 2: see original paper].

2.8 Dynamic Changes in Biomarkers During Treatment

Dynamic analysis of 20 MM patients revealed significant differences in uNGAL and uTIM-1 levels among pre-treatment MM, >PR, and relapsed MM subgroups (P<0.05). The >PR subgroup showed lower uNGAL and uTIM-1 levels than both pre-treatment and relapsed MM subgroups (P<0.05), with no significant difference between pre-treatment and relapsed subgroups (P>0.05), suggesting that uNGAL and uTIM-1 levels reflect MM disease activity [TABLE:6, FIGURE:3].

2.9 Correlation Analysis Between Biomarkers and Clinical Parameters

uNGAL, uTIM-1, sTIM-1, uVCAM-1, and urinary Activin A were positively correlated with creatinine (rs=0.498, 0.281, 0.530, 0.605, 0.301; P<0.001) and negatively correlated with eGFR (rs=-0.504, -0.302, -0.588, -0.642, -0.365; P<0.001). These biomarkers were also positively correlated with R-ISS stage (rs=0.572, 0.442, 0.471, 0.583, 0.468; P<0.001) and $ $2-microglobulin (rs=0.592, 0.584, 0.389, 0.702, 0.393; P<0.001). uNGAL, uTIM-1, sTIM-1, and urinary Activin A were positively correlated with 24-hour urinary light chain levels (rs=0.364, 0.612, 0.804, 0.631; P<0.001). uNGAL and uTIM-1 were positively correlated with clonal plasma cell percentage (rs=0.324, 0.551; P<0.001).

2.10 Diagnostic Value of Biomarkers for NDMM with KI

ROC curve analysis yielded optimal cutoff values for diagnosing MM-related KI: uNGAL 14.774 ng/mL (sensitivity=0.700, specificity=0.833, AUC=0.7954), uTIM-1 1.978 ng/mL (sensitivity=0.475, specificity=0.867, AUC=0.7092), sTIM-1 138.360 pg/mL (sensitivity=0.882, specificity=0.900, AUC=0.9146), uVCAM-1 144.400 ng/mL (sensitivity=0.947, specificity=0.625, AUC=0.9146), and urinary Activin A 33.730 pg/mL (sensitivity=0.676, specificity=0.750, AUC=0.8270) [Figure 4: see original paper]. Kaplan-Meier analysis demonstrated worse OS in patients above these cutoff values (P<0.05) [Figure 5: see original paper].

2.11 Cox Regression Analysis of All-Cause Mortality in NDMM Patients with KI

Follow-up data were collected for a median of 23 months (range 12-35 months) for 70 NDMM patients, during which 18 patients (19%) died, primarily due to MM progression (14 cases) and infection (4 cases). Univariate Cox analysis identified age, R-ISS stage, hemoglobin, creatinine, uNGAL, uTIM-1, sTIM-1, uVCAM-1, and urinary Activin A as factors influencing all-cause mortality ($P<0.05$). Multivariate Cox analysis using forward stepwise (likelihood ratio) method revealed that uNGAL and R-ISS stage were independent predictors of all-cause mortality in NDMM patients with KI ($P<0.05$) .

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3. Discussion

3.1 Elevated uNGAL, uTIM-1, uVCAM-1, and Urinary Activin A Correlate with MM Disease Progression

NGAL, a lipocalin family protein, regulates matrix metalloproteinase-9 degradation and activity, contributing to tumor progression and metastasis. TIM-1 functions as a key immune checkpoint, with studies demonstrating its overexpression in cervical cancer and promotion of tumor migration and invasion through signaling pathway regulation. VCAM-1 and Activin A are multifunctional cytokines involved in infection, immunity, kidney disease, wound repair, and tumorigenesis. Our previous research identified sTIM-1 and Activin A as participants in MM disease progression and associated pathological processes including nephropathy, anemia, and bone disease. The current study found elevated levels of uNGAL, uTIM-1, uVCAM-1, and urinary Activin A in MGRS and MM patients, with significant differences between R-ISS Stage I and Stage III, suggesting these biomarkers align with disease evolution and progression. Positive correlations between sTIM-1, urinary Activin A, and 24-hour urinary light chain levels indicate involvement in myeloma nephropathy pathogenesis. The lack of correlation with sFLC levels and sFLC k/λ may be attributed to limited clinical data and requires further validation with larger sample sizes. Similar to Du et al.' s findings identifying uNGAL as an independent predictor of MM-related KI, our results demonstrate these biomarkers correlate significantly with tumor burden markers such as $ $2-microglobulin. Additionally, uNGAL and uTIM-1 correlated positively with clonal plasma cell percentage, suggesting involvement in the MM tumor microenvironment. The positive correlation between uNGAL, uTIM-1, and cholesterol levels suggests lipids may provide energy for tumor cells during disease progression. Consistent with previous reports showing higher NGAL levels in symptomatic MM patients with advanced ISS stage, our study identified statistically significant associations between uNGAL, R-ISS stage, and all-cause mortality, highlighting NGAL' s prognostic value in MM.

3.2 Elevated uNGAL, uTIM-1, uVCAM-1, and Urinary Activin A May Be Associated with MM-Related KI

Approximately 50% of MM patients develop various forms of KI during their disease course, potentially involving the STAT1/HMGB1/TLR pathway. NGAL can induce renal tubular epithelial proliferation and activate the PI3K/Akt pathway, promoting renal inflammation and fibrosis. Inflammatory cytokines including TIM-1 participate in similar KI pathological processes. Activin A is implicated in acute and chronic KI and renal fibrosis. In our study, uNGAL, uTIM-1, sTIM-1, uVCAM-1, and urinary Activin A were elevated in MGRS and KI patients, correlating positively with creatinine and negatively with eGFR. The dynamic changes in uNGAL and uTIM-1 during treatment, with levels decreasing in the >PR subgroup and increasing again at relapse, suggest these markers reflect disease activity and treatment response. ROC analysis demonstrated high sensitivity and specificity for these biomarkers in evaluating MM-related KI.

Our findings indicate that NGAL, TIM-1, VCAM-1, and Activin A may participate in both MM disease progression and KI development within the tumor microenvironment. uNGAL and uTIM-1 may serve as early markers of tubular injury in NDMM patients, providing valuable supplements to serum creatinine for KI assessment and representing potential novel therapeutic targets to improve outcomes in MM patients.

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Author Contributions: Chen Fei and Wang Jinying performed experiments, selected study subjects, collected samples, analyzed clinical data, reviewed literature, and drafted the manuscript. Yu Haibo, Li Xin, Zhang Jiajia, Shen Man, Zhan Xiaokai, Tang Ran, Fan Sibin, Zhao Fengyi, and Zhang Tianyu assisted with data collection, statistical analysis, and figure/table preparation. Professor Huang Zhongxia guided topic selection and study design, and was responsible for quality control, manuscript revision, and overall supervision.

Conflict of Interest: The authors declare no conflict of interest.

Note: Figure translations are in progress. See original paper for figures.