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ORIGINAL RESEARCH

Expression and Clinical Significance of BCL2 Interacting Protein 3 Like (BNIP3L) in Serum of Patients with MM

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Background: BNIP3L regulates mitophagy and apoptosis, and its dysregulation is closely linked to the development and progression of cancers. Studies have shown that BNIP3L is valuable for assessing tumor progression and prognosis. This study aims to investigate the diagnostic and prognostic significance of serum BNIP3L levels in multiple myeloma (MM).

Methods: The serum level of BNIP3L were measured in 152 MM patients and 158 healthy controls by Enzyme-Linked Immunosorbent Assay (ELISA). Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic ability of MM. The prognostic relevance of serum BNIP3L levels in MM patients was assessed using Kaplan-Meier survival analysis and Cox regression.

Results: Serum BNIP3L levels were significantly elevated in MM patients compared to healthy controls ($P < 0.001$) and demonstrated significant diagnostic value (Area Under the Curve (AUC) = 0.744). Higher BNIP3L levels correlated negatively with serum calcium ($P = 0.02$) and M protein ($P = 0.03$). MM patients with extramedullary infiltration (EMI) or high-risk cytogenetic abnormalities had higher serum BNIP3L levels compared to those without these features (all $P < 0.05$). BNIP3L levels were significantly higher in non-transplant patients compared to autologous stem cell transplant (ASCT) patients ($P = 0.01$). And patients achieving Very Good Partial Response (VGPR) efficacy had significantly lower serum BNIP3L levels than those who achieving only Partial Response (PR) ($P = 0.04$). Compared to patients with low serum BNIP3L levels, those with high levels exhibited a trend toward poorer overall survival (Hazard Ratio (HR) = 1.40, 95%Confidence Interval (CI): 0.47–4.19).

Conclusion: This study identified serum BNIP3L as a potential diagnostic biomarker for active MM. Elevated BNIP3L levels were significantly associated with adverse clinical characteristics, suboptimal treatment response, and a trend toward inferior survival. Consequently, measuring serum BNIP3L could be valuable for monitoring disease progression and prognostic evaluation in MM patients.

Keywords: BNIP3L, multiple myeloma, serum biomarker, diagnosis, prognosis

Introduction

Multiple myeloma (MM) is a malignant disease characterised by the clonal proliferation of plasma cells in the bone marrow.[1] It account for approximately 1% of malignant tumours and 14% of haematological malignancies, causing up to 20% of deaths from haematological malignancies.[2,3] Abnormal proliferation of bone marrow plasma cells often results in a range of clinical symptoms in patients with MM, including hypercalcemia, renal impairment, anemia, and bone pain, known as CRAB symptoms (C: Hypercalcemia, R: Renal impairment, A: Anemia, B: Bone lesions).[4] These clinical symptoms significantly impacted the

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survival and quality of life for patients with MM. Although there are multiple effective treatment strategies for MM, including immunomodulatory drugs, proteasome inhibitors, high-dose chemotherapy combined with autologous stem cell transplantation, monoclonal antibodies, and CAR-T cell therapy, which have significantly improved patient prognosis with over 55% of patients surviving more than five years,[5] relapse at various stages of the disease course remains almost inevitable Once relapse occurs, treatment management becomes increasingly complex and challenging due to the biological heterogeneity of the disease. Therefore, monitoring disease progression during treatment and maintenance therapy using highly sensitive and specific biomarkers is crucial for timely adjustment of therapeutic strategies and for improving patient prognosis. In recent years, research on serum biomarkers for MM has made continuous progress,[6,7] with the development of novel biomarkers showing great promise. The ongoing discovery of new serum biomarkers not only contributes to earlier detection of MM but also supports personalized treatment strategies and offers new opportunities for improving clinical outcomes.

BNIP3L (Bcl-2/adenovirus E1B 19 kDa-interacting protein 3-like, also known as NIX) is a mitochondria-localized protein functionally related to Bcl-2, with critical roles in the regulation of autophagy and apoptosis.[8,9] In recent years, it has been widely recognized as a key player in tumor initiation and progression.[10] Previous studies have linked BNIP3L to cancers and reported its tumor-suppressive activity in lung cancer,[11] and breast cancer.[12] However, the role of BNIP3L varies across tumor types and exhibits a dual nature: on one hand, it acts as a tumor promoter in glioblastoma[13] and pancreatic[14] cancer by regulating mitophagy, thereby promoting tumor cell survival and progression.[8,9] On the other hand, BNIP3L loss in lung cancer is associated with chemoresistance, and its downregulation in breast cancer is closely related to increased cell invasiveness.[15,16]

Despite this established significance in solid tumor, research on BNIP3L in MM is limited. Our previous research analyzed data from the SRA, GEO, and ArrayExpress databases and found that BNIP3L mRNA expression was significantly lower in bone marrow samples from MM patients compared to healthy donors,[17] suggesting that downregulation of BNIP3L may play an important role in the occurrence and development of MM. Furthermore, our research preliminarily proved that the polymorphism of the BNIP3L gene locus rs2874670 were strongly associated with increased MM risk in the Chinese population.[18] Nevertheless, there have been no studies investigating serum BNIP3L levels in MM patients, and analyzing its clinical role in the diagnosis and prognosis of MM. This study aims to explore, for the first time, the expression levels of serum BNIP3L in MM patients and to evaluate its potential value as a diagnostic and prognostic biomarker, thereby providing a novel molecular basis for precision diagnosis and treatment of MM.

Materials and Methods

Study Subjects

This study comprised 310 subjects, 152 MM patients hospitalized in the Department of Hematology at the First Affiliated Hospital of Guangxi Medical University (Nanning, China) from January 2023 to November 2024, and 158 healthy controls. Blood samples were collected from the subjects. All MM subjects in this study met the inclusion criteria of the Chinese Guidelines for Diagnosis and Management of Multiple Myeloma (2022 revision).[19] Exclusion criteria were applied to minimize confounding factors that could influence serum biomarker levels. Specifically, patients were excluded if they had: (1) HIV, hepatitis B virus, hepatitis C virus, or other viral infections, due to the potential effects of viral infections on immune status and inflammation; (2) other neoplastic diseases, to avoid interference from additional malignancies; or (3) other hematological disorders, which could independently affect clinical and laboratory parameters. MM patients were staged using the Revised International Staging System (R-ISS),the International Staging System(ISS), and the Durie-Salmon System (DS). The healthy control group consisted of 158 individuals with no history of anemia, diabetes mellitus, inflammation, or malignancy, including 91 healthy individuals recruited from the hospital's health examination center, and 67 individuals who were either patients' family members or hospital volunteers, all of whom were confirmed eligible through health screening. The clinical information of MM patients and healthy controls in this study are presented in Table 1.

This study was approved by the ethics committee (NO.2023-E160-01). Participants gave written informed consent prior to enrollment.

Table 1 Clinical Characteristics of the Patients with MM and the Healthy Controls

Clinical Features	MM (n = 152)	Healthy Control Patients (n = 158)	*P* value
Sex, n (%)			
Male	82 (53.9)	84 (53.2)	0.89
Female	70 (46.1)	74 (46.8)	
Age, n (%)			
≤ 60y	78 (51.3)	76 (48.1)	
> 60y	74 (48.7)	82 (51.9)	
Age, mean ± SD (years)	60.1±9.3	59.9±12.6	0.57
Laboratory indices, Median (25th percentile, 75th percentile)			
Hb (g/L)	84.5 (67.6, 84.5)	144.7 (133.4, 152.0)	< 0.001
Plt (10^9/L)	175.2 (125.3, 247.0)	230.7 (200.2, 264.8)	< 0.001
Alb (g/L)	36.3 (29.6, 40.7)	44.2 (42.7, 45.9)	< 0.001
Cr (umol/L)	82.0 (63.5, 140.0)	77.0 (66.0, 87.5)	0.04
UA (μmol/L)	343.5 (255.5, 458.0)	343.0 (279.0, 407.0)	0.46
BUN (mmol/L)	5.9 (4.6, 7.8)	5.1 (4.4, 6.1)	< 0.001
CrCl (mL/min)	63.2 (41.6, 85.4)	100.3 (87.0, 112.1)	< 0.001
LDH (U/L)	183.5 (149.0, 242.0)	175.0 (155.0, 192.0)	0.08
Ca (mmol/L)	2.2 (2.1, 2.4)	2.3 (2.3, 2.4)	< 0.001
β_2-MG (μg/mL)	5.8 (3.5, 8.9)		
ESR, No. (%)			
≤40mL/min	45 (29.6)		
>40mL/min	90 (59.2)		
Plasma cells, No. (%)			
≤30%	86 (56.6)		
>30%	60 (39.5)		
Serum M protein, No. (%)			
With M protein	101 (66.4)		
Without M protein	29 (19.1)		
Urine M protein, No. (%)			
With M protein	91 (59.9)		
Without M protein	49 (32.2)		
M protein type, No. (%)			
IgG	86 (56.6)		
IgA	35 (23.0)		
IgD	9 (5.9)		
Lightchain	22 (14.5)		
FLC, No. (%)			
κ	86 (56.6)		
λ	62 (40.8)		
DS stage, No. (%)			
I	6 (3.9)		
II	8 (5.3)		
III	132 (86.8)		
ISS stage, No. (%)			
I	30 (19.7)		
II	13 (8.6)		
III	92 (60.5)		
R-ISS stage, No. (%)			
I	8 (5.3)		
II	49 (32.2)		
III	63 (41.4)		

(*Continued*)

Table 1 (Continued).

Clinical Features	MM (n = 152)	Healthy Control Patients (n = 158)	*P* value
Fluorescence In Situ Hybridization(FISH), No. (%)			
Positive	91 (59.9)		
Negative	42 (27.6)		
Genetic risk type, No. (%)			
High-risk	73 (48.0)		
Standard-risk	35 (23.0)		
Extramedullary infiltration (EMI), No. (%)			
With EMI	25 (16.4)		
Without EMI	118 (77.6)		
OLs, No. (%)			
No OLs	61 (40.1)		
Fewer than 3 OLs	24 (15.8)		
More than 4 OLs	59 (38.1)		
Autologous stem cell transplantation, No. (%)			
With Transplant	10 (7.3)		
Without Transplant	137 (90.1)		
Chemotherapy, No. (%)			
With Chemotherapy	56 (36.8)		
Without Chemotherapy	50 (32.9)		
Therapeutic effect, No. (%)			
VGPR	19 (12.5)		
PR	12 (7.9)		
PD	15 (9.9)		

Notes: Patients without relevant clinical data were excluded from the respective subgroup analyses. Therefore, the sum of patients across subgroups may not equal the total cohort.

Abbreviations: Hb, hemoglobin; Plt, platelet; Alb, albumin; Cr, creatinine;UA,uric acid; BUN, blood urea nitrogen; CrCl, creatine clearance; LDH, lactate dehydrogenase; Ca, calcium; β_2-MG, β_2-microglobulin; ESR, erythrocyte sedimentation rate; FLC, free light chain; DS, Durie salmon staging system; ISS, international staging system; R-ISS, revised international staging system; OLs, osteolytic Lesions; VGPR, very good partial response; PR, partial response; PD, progressive disease; MM, multiple myeloma.

Data Collection from Study Subjects

The basic information of MM patients participated in the study, including admission age, gender, DS, ISS and R-ISS staging were obtained. Clinical examination results of patients upon initial diagnosis at our hospital were collected, including serum hemoglobin (Hb), platelets (Plt), albumin (Alb), lactate dehydrogenase (LDH), uric acid (UA), blood urea nitrogen (BUN), creatinine (Cr), Creatinine Clearance (CrCl), β2-microglobulin (β2-MG), calcium (Ca), erythrocyte sedimentation rate (ESR), light chain κ/λ ratio, and Monoclonal protein (M protein) type, Fluorescence in Situ Hybridization (FISH). Additionally, EMI and osteolytic lesions (OLs) in MM patients were recorded upon admission based on CT, PET/CT, MRI and other imaging results. At the same time, the chemotherapy regimen used and the ASCT status of patients were recorded in their electronic medical records when they were first admitted to our hospital. Patients without relevant clinical data were excluded from the respective subgroup analyses. Therefore, the sum of patients across subgroups may not equal the total cohort. Clinical parameters are grouped following the Guidelines for the diagnosis and management of MM in China (2022 revision).[19]

Serum BNIP3L Level Detection

An ELISA-based quantification of BNIP3L levels in blood samples of MM patients and healthy controls was carried out using a sandwich-type assay kit (Human BNIP3L ELISA Kit, Cat. Nos. MM-622688H1 and MM-622688H2. Jiangsu Enzyme Immunity Industry Co., Ltd, Jiangsu, China). The operation steps strictly follow the manufacturer's guidelines.

Follow-Up of the Enrolled Patients

Due to the drop out and exclusion criteria of some MM patients, the clinical data and the overall survival (OS) time of only 130 MM patients were collected from initial diagnosis to the study's follow-up completion on July 1, 2025. Among them, patients with outpatient and inpatient records in our hospital in the past 3 months are considered as survival as appropriate. Patients were excluded if they met any of the following criteria: 1) smoking, as smoking can affect immune function and biomarker levels; 2) taking medications that could influence glucose levels for at least three months prior to recruitment (patients using folic acid, iron, vitamin D, or iodine supplements were not excluded), since altered glucose metabolism might impact disease progression and biomarker expression; 3) a history of cardiovascular disease, which may affect overall prognosis independently of MM; 4) pregestational diabetes mellitus, due to its impact on metabolic and inflammatory states that could interfere with study outcomes; or 5) having multiple pregnancies, as hormonal and immune changes associated with multiple pregnancies could influence biomarker levels and disease progression.

Statistical Analysis

Statistical analysis was conducted using the social science statistical software package (SPSS 26.0, IBM Corp, Armonk, NY, USA). Mann–Whitney *U*-test or Wilcoxon signed-rank test was applied to evaluate differences in continuous variables between the two groups. Categorical variables were analyzed using the chi-square test. Receiver Operating Characteristic (ROC) curve analysis was carried out to derive ROC curves and calculate the area under the curve (AUC) for the diagnostic parameters. This analysis served to identify the optimal cutoff value for serum BNIP3L levels. DeLong method[20] was used to compare the AUC values of the ROC curves to assess the diagnostic performance of each parameter for MM. MM patients were categorized into high and low serum BNIP3L levels with these cutoff values. Overall survival was analyzed utilizing the Kaplan-Meier method. Log-Rank (Mantel-Cox) test was utilized to evaluate divergences in survival curves. $P < 0.05$ was considered statistically significant.

Result

Comparison of Serum BNIP3L Levels Between MM Patients and Healthy Individuals

The serum BNIP3L levels of 152 MM patients and 158 healthy individuals were measured by ELISA. The results demonstrated that the median serum BNIP3L concentration in MM patients was significantly higher than those in healthy individuals (Figure 1, median = 1710.66 pg/mL and 1381.50 pg/mL, respectively; $P < 0.001$).

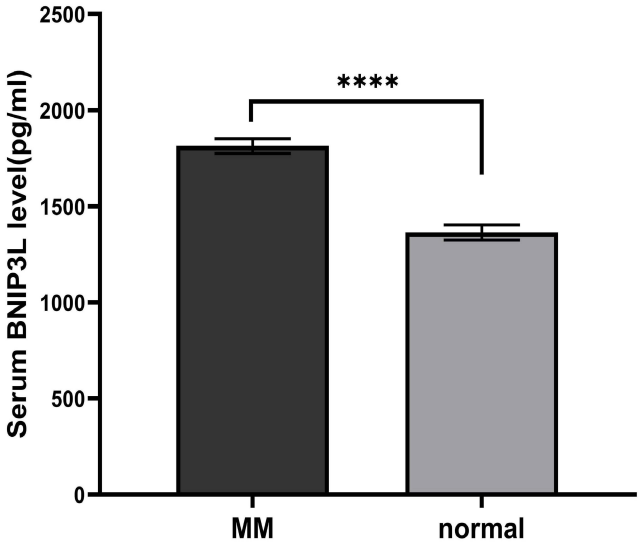

Figure 1 Comparison of serum BNIP3L levels between MM patients and healthy individuals. (****$P < 0.0001$).

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Correlation Between Different Clinical Parameters and Serum BNIP3L Levels

To determine whether serum BNIP3L level exhibits a synchronous linear variation with disease-related parameters and explore its continuous quantitative characteristics during disease progression, we performed a linear correlation analysis between serum BNIP3L levels and other laboratory parameters. These parameters include Hb, Plt, Alb, UA, BUN, β2-MG, LDH, Cr, CrCl, ESR, Ca, M protein, marrow plasma cells, free lightchain and immunoglobulin subtype. The Mann–Whitney *U*-test demonstrated that serum BNIP3L levels were significantly associated with both Ca ($P = 0.01$) and M protein levels ($P = 0.03$) in MM patients (Figures 2A and B). The results showed that as the serum BNIP3L level of MM patients increased, their serum Ca and M protein level gradually decreased. In addition, we found that with the serum level of BNIP3L increased in patients with MM, the CrCl level gradually decreased (Figure 2C, $P = 0.32$), while the LDH level increased (Figure 2D, $P = 0.09$). However, neither trend was statistically significant. The levels of BNIP3L between different groups of other related parameters was compared. The results showed that patients with serum M protein had significantly higher BNIP3L level than patients without M protein ($P < 0.001$), and the similar results were

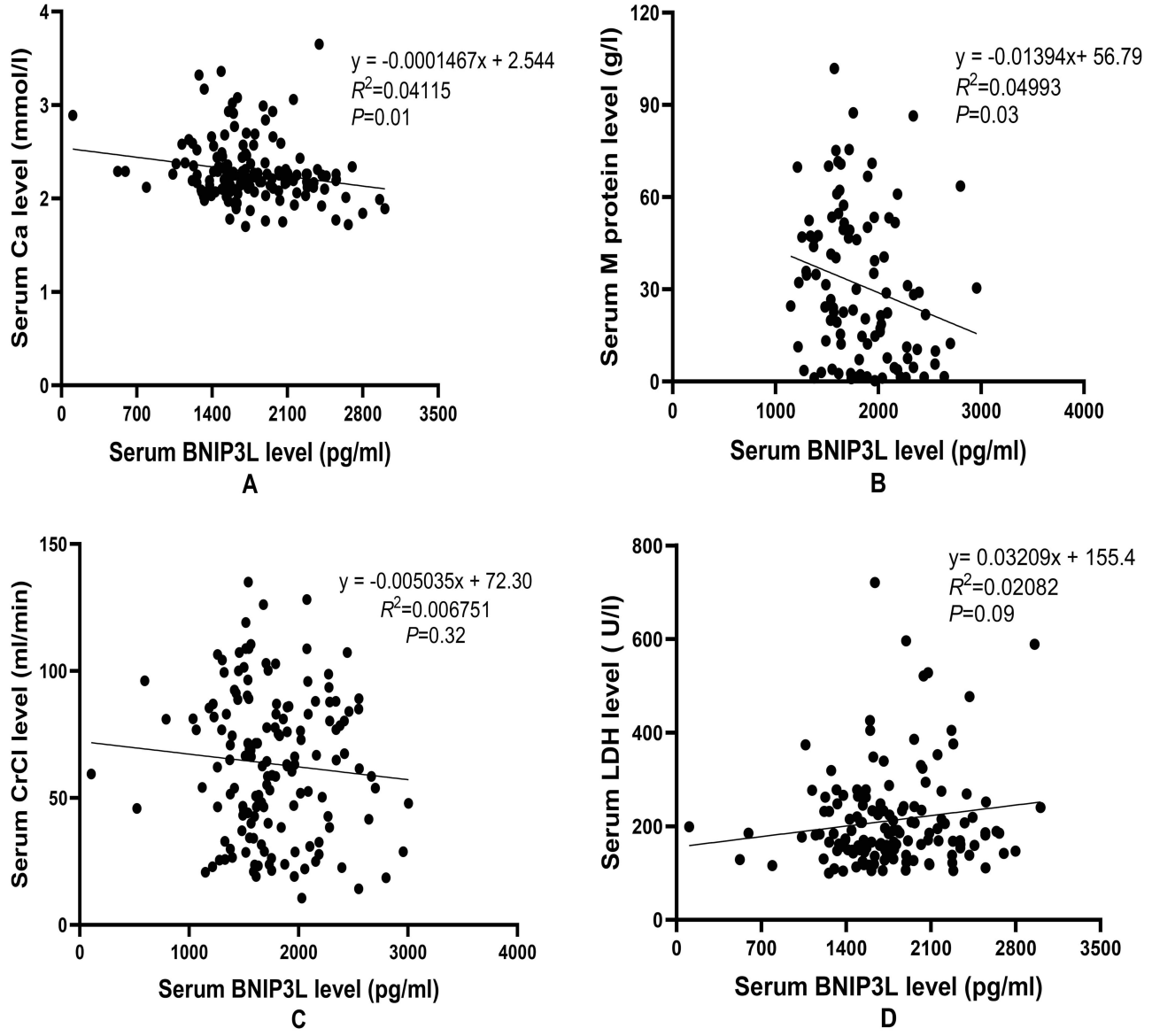

Figure 2 (**A**) Correlation between serum BNIP3L and Ca levels in MM patients. (**B**) Correlation between serum BNIP3L and M protein levels in MM patients. (**C**) Correlation between serum BNIP3L and CrCl levels in MM patients. (**D**) Correlation between serum BNIP3L and LDH levels in MM patients.

obtained after grouping analysis of urinary M protein ($P = 0.04$). In addition, we also found that patients with high levels of Cr ($P = 0.07$), Alb ($P = 0.06$), UA ($P = 0.07$), and ESR ($P = 0.08$) had higher BNIP3L level than those with low level. However, these four differences were not statistically significant. The analytical results are summarized in Table 2. To further evaluate the prognostic significance of elevated serum BNIP3L levels, we performed univariate and multivariate Cox regression analyses, with a high level of BNIP3L as the outcome variable. Univariate analysis identified several

Table 2 Comparison of Serum BNIP3L Levels Between Different Clinical Parameter Groups in MM Patients

Parameters	BNIP3L (pg/mL)	z	*P* value
Gender			
Male	1755.16	−1.662	0.10
Female	1628.00		
Age (y)			
≤60	1681.34	−0.267	0.79
>60	1714.20		
Hb (g/L)			
≤100	1687.99	−0.076	0.94
>100	1737.14		
Plt (10^9/L)			
≤100	1712.57	−0.126	0.90
>100	1706.20		
β_2-MG (mg/L)			
≤5.5	1702.89	−0.898	0.37
>5.5	1716.31		
Ca (mmol/L)			
≤2.75	1710.48	−0.801	0.42
>2.75	1680.52		
Cr (mg/dL)			
≤177	1649.54	−1.847	0.07
>177 L	1815.01		
CrCl (mL/min)			
≤40	1721.79	−0.474	0.64
>40	1692.98		
Alb (g/L)			
≤35	1615.63	−1.902	0.06
>35	1722.66		
LDH (U/L)			
≤220	1687.99	−0.562	0.57
>220	1710.83		
UA (μmol/L)			
≤420	1623.82	−1.832	0.07
>420	1742.63		
BUN (mmol/L)			
≤7.1	1660.64	−0.577	0.56
>7.1	1732.84		
ESR (mm/60min)			
≤40	1609.00	−1.781	0.08
>40	1710.83		
Plasma cell (%)			
≤30	1709.50	−1.036	0.30
>30	1637.63		

(*Continued*)

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Table 2 (Continued).

Parameters	BNIP3L (pg/mL)	z	*P* value
Serum M protein			
With M protein	1785.41	−3.311	<0.001
Without M protein	1531.30		
Urine M protein			
With M protein	1717.56	−2.012	0.04
Without M protein	1565.50		
M protein type			
IgG	1722.23	1.833	0.61
IgA	1638.43		
IgD	1841.85		
Lightchain	1580.66		
Free Light Chain			
κ	1719.68	−0.389	0.70
λ	1709.99		
DS			
I and II	1537.18	−2.097	0.04
III	1714.20		
ISS			
I and II	1609.00	−1.507	0.13
III	1744.78		
R-ISS			
I and II	1635.91	−1.471	0.14
III	1721.79		
FISH			
Positive	1791.06	−2.277	0.02
Negative	1670.13		
Genetic risk type			
High-risk	1789.65	−2.488	0.01
Standard-risk	1612.39		
OLs			
No OLs	1612.39	2.048	0.36
Fewer than 3 OLs	1713.53		
More than 4 OLs	1755.09		
EMI			
With EMI	1814.99	−2.750	0.01
Without EMI	1619.82		
Autologous stem cell transplantation			
With Transplant	1489.15	−2.535	0.01
Without Transplant	1722.66		
Chemotherapy			
With Chemotherapy	1665.51	−1.884	0.06
Without Chemotherapy	1847.12		
Therapeutic effect			
VGPR	1531.30	6.439	0.04
PR	1870.30		
PD	1687.99		

Abbreviations: Hb, hemoglobin; Plt, platelet; Alb, albumin; Cr, creatinine; UA, uric acid; CrCl, endogenous creatinine clearance; LDH, lactate dehydrogenase; Ca, calcium; β_2-MG, β_2-microglobulin; ESR, erythrocyte sedimentation rate; DS, Durie salmon staging system; ISS, international staging system; R-ISS, revised international staging system; FISH, fluorescence in situ hybridization; EMI, extramedullary infiltration; OLs, osteolytic Lesions; VGPR, very good partial response; PR, partial response; PD, progressive disease; MM, multiple myeloma.

clinical parameters potentially associated with high BNIP3L levels. Subsequent multivariate Cox regression analysis, which adjusted for potential confounding factors, demonstrated that β_2-MG was an independent factor significantly associated with high serum BNIP3L levels (HR = 1.119, 95% CI: 1.029–1.195, P = 0.007). Although other parameters such as age, Hb, Alb, Cr, UA, CrCl, plasma cell, and FISH showed associations in prior analyses, they did not retain independent significance in the final multivariate model for predicting high BNIP3L levels. These finding reinforce the specific and independent relationship between β_2-MG and elevated BNIP3L, underscoring its potential role in the underlying disease pathophysiology. The results are shown in the forest plot (Figure 3), with data summarized in Table 3.

Correlation of Serum BNIP3L Levels Among MM Patients with Different Clinicopathological Features

To evaluate the association between the serum BNIP3L levels and clinicopathological features in MM patients, we collected some relevant clinical data, including the presence of OLs, EMI, and FISH results. Among all enrolled patients, 25 (16%) patients had EMI. Group comparisons indicated that the serum levels of BNIP3L in patients with EMI were significantly higher than in those without EMI (Figure 4A, P = 0.01). In addition, we collected the FISH results from the enrolled patients, among whom 91 (59.9%) showed abnormal findings. The abnormal results were mainly divided into 1q21 amplification, t(4;14), t(14;16), del(13q), and del(17p). The most common abnormality was 1q21 amplification. We compared the serum level of BNIP3L between MM patients with abnormal and normal FISH results. Results showed that the serum level of BNIP3L in patients with abnormal FISH results was markedly increased compared to those with normal FISH results (Figure 4B, P = 0.02). A detailed comparison of serum BNIP3L levels was performed among three groups: those with normal FISH results, those with standard-risk (SR) genetic abnormalities, and those with high-risk (HR) genetic abnormalities. It indicated that the serum level of BNIP3L in patients with HR genetic abnormalities were significantly increased compared to those without abnormal genetic abnormalities (Figure 4C, P =0.01). Furthermore, serum levels of BNIP3L in patients carrying any of the three common HR genetic abnormalities (t(4;14), del(17p), and 1q21) in this study were compared (Figure 4D). But none of these comparisons revealed statistically significant differences (all $P > 0.05$). Among the patients, 61 (40.1%) had no osteolytic lesions (OLs), 24 (15.8%) had fewer than 3 OLs, and 59 (38.8%) had more than 4 OLs throughout the body. Based on these findings, patients were categorized into three groups: no OLs, fewer than 3 OLs, and more than 4 OLs. Then we compared the variation in serum levels of

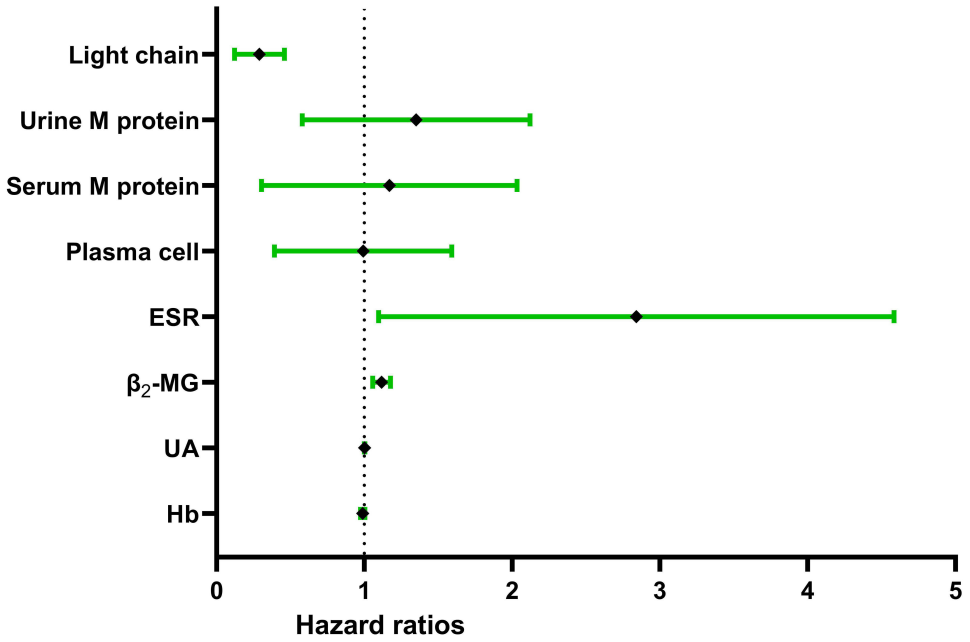

Figure 3 Forest plot of multivariable Cox regression. The solid vertical line indicates no association (HR = 1), horizontal bars depict 95% confidence intervals (CIs). The outcome was defined as "high-level BNIP3L" (BNIP3L expression above the optimal cut-off).

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Table 3 Univariable and Multivariable Cox Regression Analyses of Factors Associated with BNIP3L Elevation

Variable	Univariable HR (95% CI)	*P* value	Multivariable HR (95% CI)	*P* value
Gender	1.143 (0.774–1.688)	0.500		
Age	0.995 (0.976–1.014)	0.584		
Hb	0.985 (0.978–0.993)	<0.001	0.989 (0.974–1.004)	0.134
Plt	0.999 (0.997–1.002)	0.614		
Alb	0.985 (0.961–1.009)	0.209		
Cr	1.001 (1.000–1.002)	0.089		
UA	1.002 (1.001–1.003)	0.002	1.001 (0.999–1.0003)	0.591
BUN	1.026 (0.991–1.062)	0.146		
CrCl	0.994 (0.988–1.000)	0.060		
LDH	1.000 (1.000–1.001)	0.112		
Ca	1.242 (0.610–2.530)	0.551		
β_2-MG	1.074 (1.039–1.109)	<0.001	1.116 (1.057–1.178)	<0.001
ESR	2.226 (1.388–3.571)	<0.001	2.483 (1.302–4.735)	0.006
Plasma cell	1.895 (1.247–2.878)	0.003	0.871 (0.461–1.643)	0.669
Serum M protein	2.296 (1.370–3.845)	0.002	0.956 (0.431–2.120)	0.911
Urine M protein	1.881 (1.247–2.837)	0.003	1.204 (0.664–2.183)	0.541
Light chain	0.568 (0.380–0.850)	0.006	0.257 (0.140–0.473)	<0.001

Abbreviations: CI, confidence interval; HR, hazard ratio; Hb, hemoglobin; Plt, platelet; Alb, albumin; Cr, creatinine; UA, uric acid; BUN, blood urea nitrogen; CrCl, creatine clearance; LDH, lactate dehydrogenase; Ca, calcium; β_2-MG, β_2-microglobulin; ESR, erythrocyte sedimentation rate.

BNIP3L among the three groups and investigated whether BNIP3L serum levels were associated with the extent of OLs of MM patients. However, the results showed no significant correlation between serum levels of BNIP3L and the patients' osteolytic lesions. (Table 2, $P > 0.05$).

Correlation Analysis of Serum Levels of BNIP3L in MM Patients with Different Treatment Interventions and Efficacy Responses

To investigate the relationship between serum BNIP3L levels and treatment interventions and response outcomes in MM patients, we conducted a subgroup analysis based on chemotherapy status, ASCT status, and depth of treatment response. Among the 152 enrolled patients, 66 (43.4%) had received chemotherapy, while 50 (32.9%) not. Intergroup analysis revealed that serum levels of BNIP3L were lower in patients who received chemotherapy compared to those who did not. Unfortunately, this difference was not statistically significant ($P = 0.06$). Further subgroup analysis based on ASCT status showed that 10 (6.8%) patients underwent transplantation, while 137 (91.3%) patients did not. Results indicated that BNIP3L levels were significantly higher in non-transplant patients compared to transplant patients (Figure 4E, $P = 0.01$). Regarding treatment efficacy, patients were categorized into three groups according to the International Myeloma Working Group (IMWG) criteria: VGPR (very good partial response, n=19, 12.5%), PR (partial response, n=12, 7.9%), and PD (disease progression, n=15, 9.9%). Stratified efficacy comparisons showed that serum BNIP3L levels were significantly higher in the PR group than in the VGPR group (Figure 4F, $P = 0.04$).

Diagnostic Value of Serum Level of BNIP3L in MM

ROC analysis was performed using the ROC curve with serum BNIP3L concentrations from 152 MM patients and 158 healthy controls (Figure 5A). The analysis demonstrated that serum BNIP3L levels effectively differentiate MM patients from healthy control (AUC = 0.744, Youden index = 0.39, cut-off value = 1443.72). It showed a good diagnostic value of BNIP3L for MM patient. Simultaneously, we constructed ROC curves for these parameters from both MM patient group and healthy control group and the, including Hb, Plt, Alb, Cr, BUN, CrCl, and Ca (AUC = 0.950, 0.686, 0.870, 0.569, 0.635, 0.834, and 0.665, respectively) (Figure 5B–H). Finally, to enhance diagnostic accuracy, the

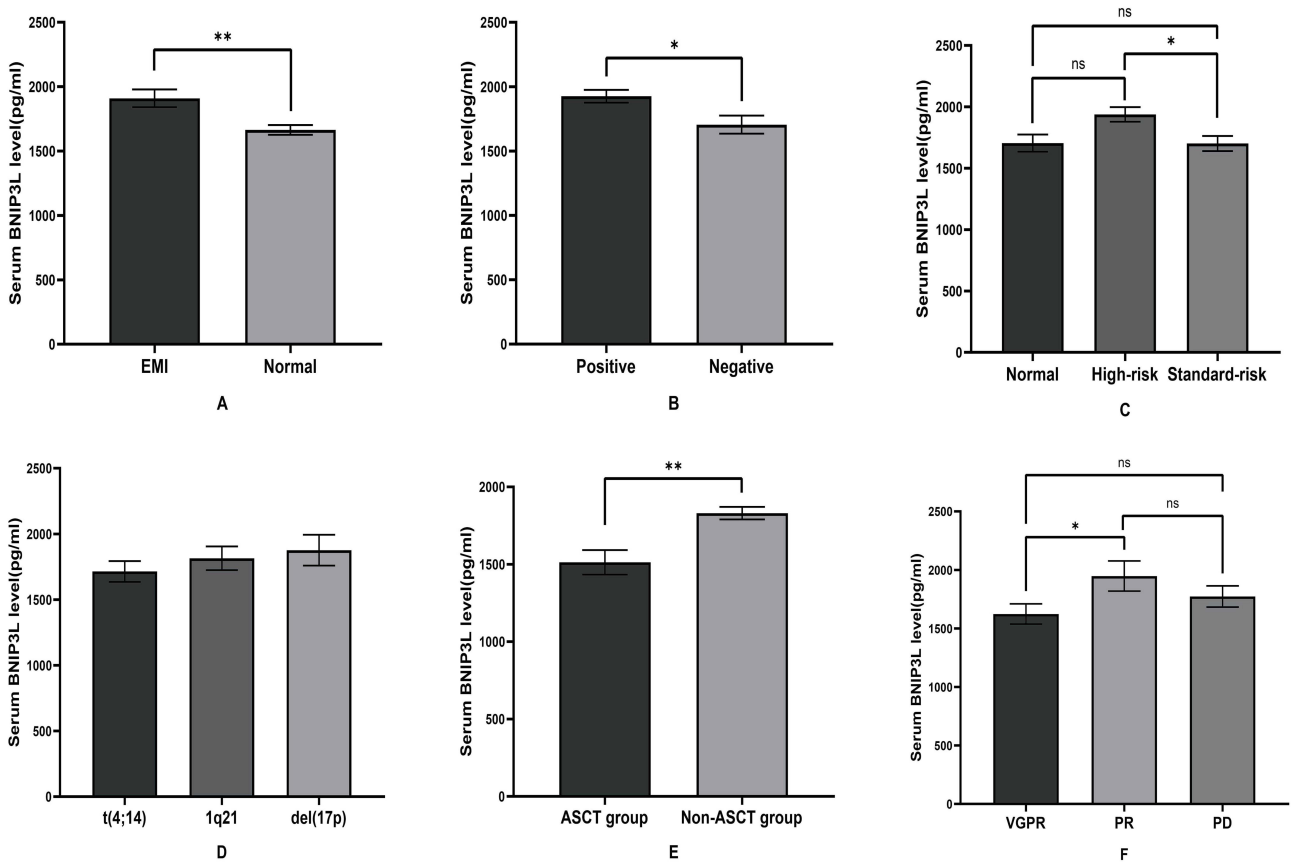

Figure 4 (**A**) Comparison of serum BNIP3L levels between MM patients with and without EMI. (**$P < 0.01$). (**B**) Comparison of serum BNIP3L levels between MM patients with negative FISH results and those with positive results. (*$P < 0.05$). (**C**) Comparison of serum BNIP3L levels among Normal, High-risk, and standard-risk groups. (*$P < 0.05$). (**D**) Comparison of serum BNIP3L levels among MM patients with different high-risk genetic abnormalities. (**E**) Comparison of serum BNIP3L levels between the ASCT group and Non-ASCT group in MM patients. (**$P < 0.01$). (**F**) Comparison of serum BNIP3L levels among MM patients with VGPR, PR, and PD after chemotherapy. (*$P < 0.05$).

combined diagnostic curve was obtained by integrating BNIP3L and the above clinical parameters (AUC = 0.970) (Figure 5I), compare to the above clinical parameters (AUC = 0.967) (Figure 5J). The comparison between the combined diagnostic ROC curve and the ROC curves of individual parameters is shown in Figure 6, with data summarized in Table 4.

Survival Analysis

Among the patients who were followed up, 20 (13%) patients died from MM. The median follow-up time for the 130 patients was 22.5 months. Based on the ROC-derived cut-off value, we divided 130 patients into low level groups and high level groups of serum BNIP3L. The median follow-up times were 24.6 months (range, 8.6 to 97.5 months) for the low level groups and 21.7 months (range, 0.3 to 102.6 months) for the high level groups. Kaplan-Meier curves were utilized to compare overall survival (OS) between these two groups. The results demonstrated a trend toward poorer survival in the high BNIP3L level group compared to the low BNIP3L level group, with a hazard ratio (HR) of 1.40 (95% CI: 0.47–4.19). By the end of follow-up, the mortality rates were 2.0% in the low BNIP3L level group and 11.2% in the high BNIP3L level group. However, despite the observed trend, the difference in OS did not reach statistical significance (Figure 7, $P = 0.55$). To further identify independent prognostic factors for OS, a multivariable Cox regression analysis was performed. Several clinical parameters were significantly associated with overall survival. Specifically, older age (HR = 1.327, 95% CI: 1.042–1.688, $P = 0.022$) and higher serum Cr levels (HR = 1.018, 95% CI: 1.001–1.034, $P = 0.032$) were identified as significant risk factors for poorer survival. Conversely, higher Hb levels (HR = 0.881, 95% CI: 0.800–0.970, $P = 0.010$) were associated with a favorable prognosis. Importantly, when adjusted

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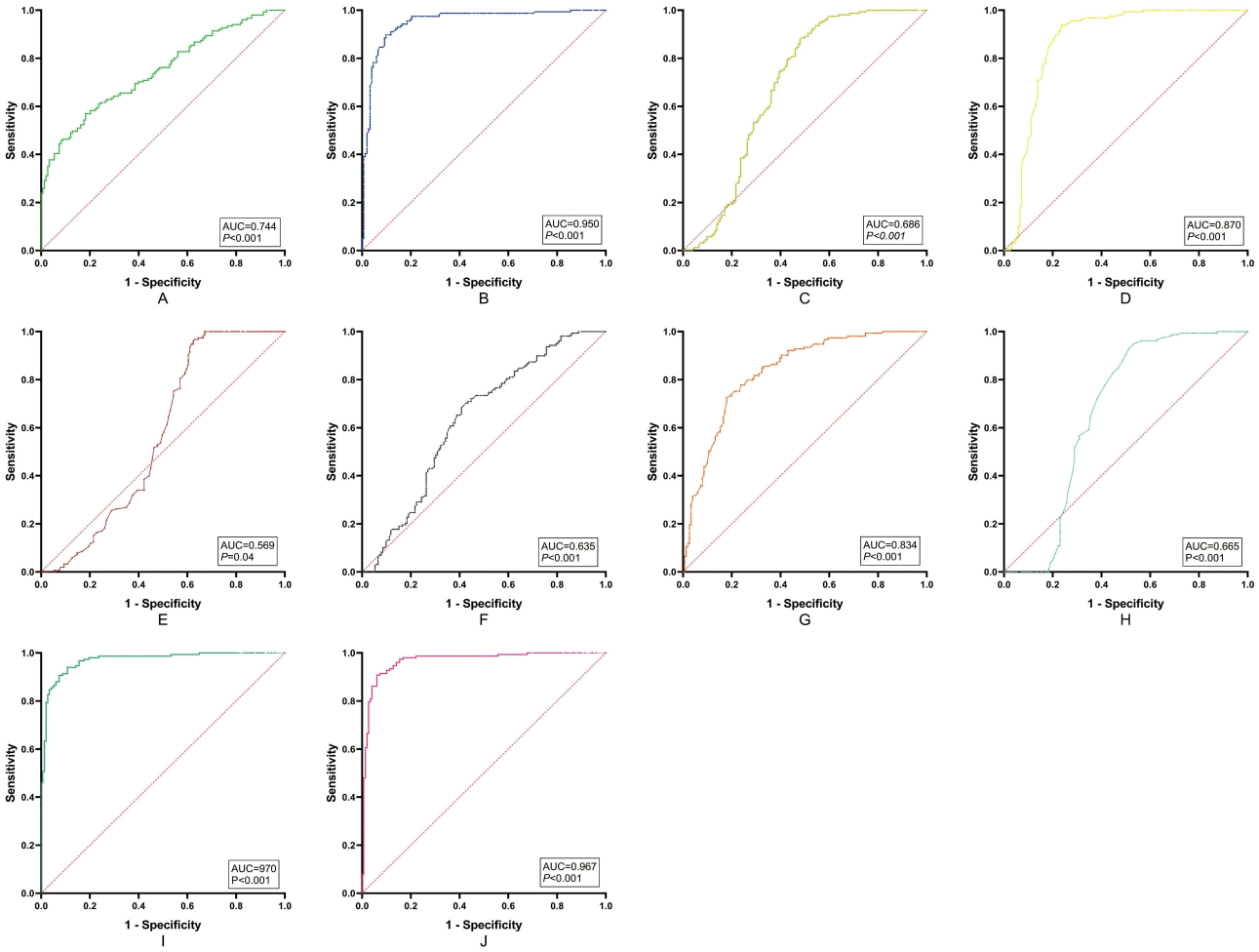

Figure 5 Receiver operating characteristic (ROC) curves analysis of MM patients and healthy controls: BNIP3L (**A**) Hb (**B**) Plt (**C**) Alb (**D**) Cr (**E**) BUN (**F**) CrCl (**G**) and Ca (**H**). (**I**) ROC curve analysis of serum BNIP3L levels in combination with Hb, Plt, Alb, Cr, BUN, CrCl, and Ca in MM and healthy controls. (**J**) ROC curve analysis of the combination of Hb, Plt, Alb, Cr, BUN, CrCl, and Ca in MM patients and healthy controls.

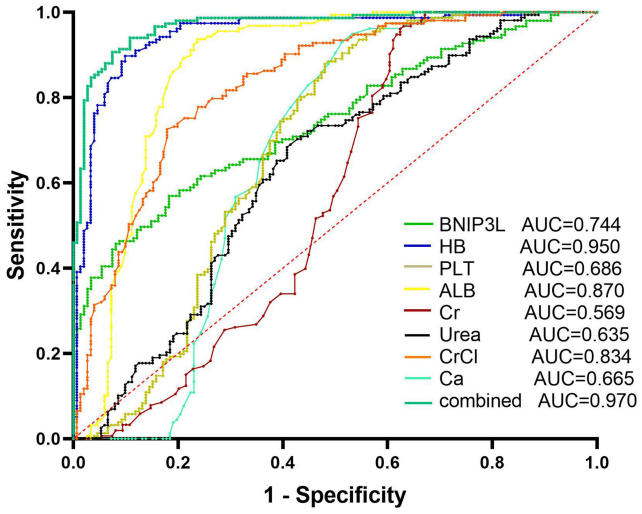

Figure 6 Receiver operating characteristic (ROC) curve analysis of the diagnostic efficacy of a combined model compared to individual parameters.

Table 4 Comparison of ROC Curves Between General Biochemical Parameters Related to the Diagnosis of MM

Variable	AUC	SE[20]	95% CI[21]	*P*
Combined	0.970	0.0088	0.953 to 0.987	<0.001
BNIP3L	0.744	0.0281	0.689 to 0.799	<0.001
Hb	0.950	0.0127	0.925 to 0.975	<0.001
Plt	0.686	0.0320	0.623 to 0.748	<0.001
Alb	0.870	0.0228	0.826 to 0.915	<0.001
Cr	0.569	0.0349	0.501 to 0.638	0.04
UA	0.518	0.0340	0.452 to 0.585	0.58
BUN	0.635	0.0320	0.572 to 0.697	<0.001
CrCl	0.834	0.0232	0.788 to 0.879	<0.001
LDH	0.563	0.0353	0.493 to 0.632	0.06
Ca	0.665	0.0338	0.599 to 0.731	<0.001

Abbreviations: AUC, area under the curve; BNIP3L, Bcl 2 interacting protein 3-like; Hb,hemoglobin; Plt, platelet; Alb, albumin; Cr, creatinine; BUN, blood urea nitrogen; CrCl, endogenous creatinine clearance; Ca, calcium; LDH, lactate dehy-drogenase; Combined, combined ROC curve for BNIP3L, Hb, Plt, Alb, Cr, BUN, CrCl, and Ca.

for these covariates in the multivariate model, serum BNIP3L level emerged as an independent prognostic factor, with higher levels conferring an increased risk of mortality (HR = 1.004, 95% CI: 1.001–1.007, $P = 0.021$). The results are shown in the forest plot (Figure 8), with data summarized in Table 5.

Discussion

MM is a complex hematologic malignancy marked by clonal plasma cell expansion and diverse clinical manifestations.[22] Given its heterogeneity and incurability,[23] there is a pressing need for novel, non-invasive serum biomarkers to improve diagnosis and prognosis. BNIP3L, a protein involved in mitochondrial autophagy and tumor progression,[8,9,14,24] has been linked to cancers,[25–28] with prior data showing its decreased mRNA expression in MM bone marrow[17] and genetic variants associated with increased disease risk.[18] And it is well known that biomarker levels can be regulated differently in cellular transcriptomes compared to systemic circulation. In this context, the contrast between elevated serum BNIP3L levels and its downregulated mRNA expression in the bone marrow microarrays highlights the compartment-specific

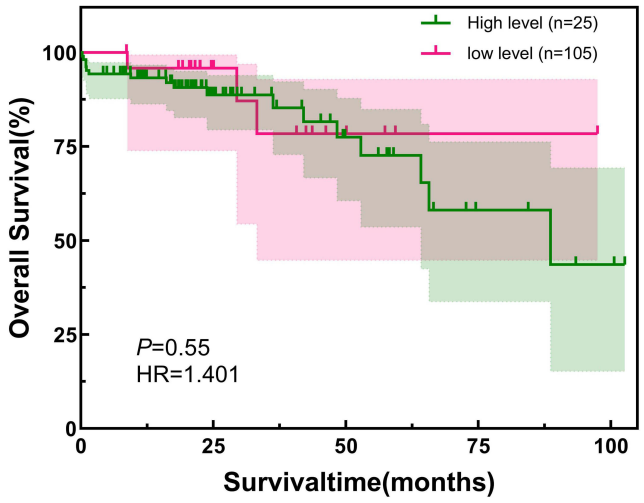

Figure 7 Serum BNIP3L levels in relation to survival analysis of MM patients.

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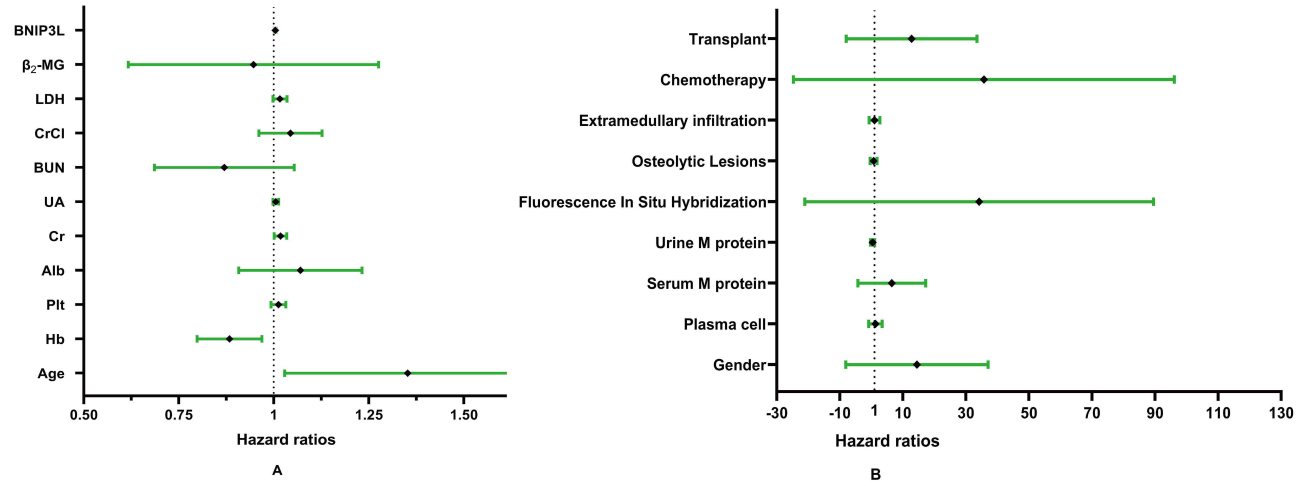

Figure 8 (**A**) Forest plot of multivariable Cox proportional hazards regression analysis for continuous clinical parameters. (**B**) Forest plot of multivariable Cox proportional hazards regression analysis for categorical clinical parameters. The solid vertical line indicates no association (HR = 1), horizontal bars depict 95% confidence intervals (CIs). The outcome variables are patient survival status and overall survival time.

regulation of BNIP3L in multiple myeloma. The decreased mRNA reflects the transcriptome within malignant plasma cells, whereas the elevated serum protein may originate from non-malignant cells[29,30] or result from altered post-transcriptional regulation in the tumor microenvironment.[31] Thus, serum BNIP3L emerges as a novel, non-invasive biomarker that captures the systemic physiological response to MM, which is distinct from its cell-intrinsic role within the tumor clone. However, the clinical relevance of serum levels of BNIP3L in MM remained unclear. Our study

Table 5 Cox Proportional Hazards Regression Analysis of Overall Survival

Variable	HR	Lower 95% CI	Upper 95% CI	*P*
Gender	2.681	0.177	40.512	0.477
Age	1.327	1.042	1.688	0.022
Hb	0.881	0.800	0.970	0.010
Plt	1.012	0.993	1.032	0.219
Alb	1.062	0.912	1.236	0.441
Cr	1.018	1.001	1.034	0.032
UA	1.005	0.998	1.013	0.165
BUN	0.857	0.693	1.06	0.156
CrCl	1.042	0.962	1.128	0.313
LDH	1.016	0.998	1.035	0.087
β_2-MG	0.909	0.638	1.293	0.595
BNIP3L	1.004	1.001	1.007	0.021
Plasma cell	0.120	0.004	3.796	0.229
Serum M protein	0.544	0.016	18.949	0.737
Urine M protein	0.067	0.004	1.163	0.063
FISH	4.437	0.201	98.065	0.346
OLs	0.205	0.021	1.907	0.170
EMI	0.048	0.001	2.986	0.150
Chemotherapy	1.690	0.027	105.479	0.803
Transplant	1.540	0.065	36.727	0.790

Abbreviations: CI, confidence interval; HR, hazard ratio; Hb, hemoglobin; Plt, platelet; Alb, albumin; Cr, creatinine; UA,uric acid; BUN, blood urea nitrogen; CrCl, creatine clearance; LDH, lactate dehydrogenase; β_2-MG, β_2-microglobulin; FISH, fluorescence in situ hybridization; OLs, osteolytic Lesions; EMI, extramedullary infiltration.

compared the differences of serum levels of BNIP3L between MM patients and healthy controls and analyzed their associations with clinical parameters and survival outcomes.

Our findings show that serum BNIP3L levels are significantly elevated in MM patients compared to healthy individuals ($P < 0.001$). ROC curve analysis demonstrated a good diagnostic value of BNIP3L for MM patient (AUC = 0.744, Youden index = 0.39). As well, the study also found that the serum level of BNIP3L was negatively correlated with concentration of serum Ca (R = −0.186, $P = 0.01$) and M protein (R = −0.258, $P = 0.03$), and was significantly elevated in both serum and urine M protein-positive subgroups ($P < 0.001$; $P = 0.04$). Further multivariate Cox regression analysis reinforced the specific association of BNIP3L with disease pathophysiology, identifying β_2-MG as an independent factor significantly linked to high serum BNIP3L levels (HR = 1.119, 95% CI: 1.029–1.195, $P = 0.007$). These results suggest that BNIP3L may serve as a complementary biomarker to improve the overall accuracy of MM diagnostic models or for early risk stratification of patients. Meanwhile, the negative correlation with key disease indicators also suggests that BNIP3L may act as a novel independent prognostic factor, and future research could verify whether it can help identify high-risk or low-risk patient groups that standard risk models are unable to discern. In recent years, serum biomarkers such as β2-MG, LDH, CRP, albumin, and sFLCs have been widely applied in the diagnosis, prognostication, and risk classification MM.[32] While these markers largely reflect overall tumor burden, systemic inflammation, or renal function, and may not capture specific molecular pathways driving disease progression. BNIP3L, a mitochondrial protein involved in regulating mitophagy, represents a mechanistically distinct marker that links mitochondrial homeostasis and cellular stress response to MM pathobiology.[33,34] In this study, elevated serum BNIP3L levels were associated with poorer prognosis, providing prognostic information that complements traditional biomarkers. Furthermore, BNIP3L levels negatively correlated with serum Ca and M protein concentration, indicating potential involvement in tumor cell survival and calcium metabolism. These findings suggest that BNIP3L may serve as an independent biomarker with added prognostic value, particularly in cases where conventional indicators offer limited insight. Incorporating BNIP3L into existing prognostic frameworks could refine risk stratification and support more personalized treatment strategies in MM.

Stratified analyses revealed that elevated BNIP3L levels were associated with several clinical features, including positive M protein status, presence of EMI, high-risk chromosomal abnormalities detected by FISH (eg, 1q21 amplification, 17p deletion), absence of ASCT, and certain disease stages or treatment response groups. Notably, patients with EMI or high-risk cytogenetic abnormalities showed significantly higher BNIP3L levels, suggesting a link between BNIP3L and increased tumor aggressiveness and poor prognosis in MM.[35,36]

In terms of treatment response, serum BNIP3L levels decreased significantly following ASCT. Additionally, patients achieving VGPR showed lower BNIP3L levels than those with only PR, indicating that BNIP3L might serve as a dynamic biomarker for reflecting treatment sensitivity and disease remission status. These observations align with previous studies showing that BNIP3L expression is responsive to cellular stress, including chemotherapy and hypoxia, with levels fluctuating in accordance with stress intensity.[31]

Multivariate analysis demonstrated that combining BNIP3L with clinical conventional parameters (including Hb, Plt, Alb, Cr, BUN, CrCl, and Ca) significantly improved diagnostic accuracy for MM (AUC = 0.970), compare to only BNIP3L (AUC = 0.744) or those integrating clinical parameters (AUC = 0.967). This suggests that BNIP3L may complement traditional markers, particularly in early-stage or atypical cases where conventional diagnostic sensitivity is limited.

Survival analysis preliminary indicated a trend toward poorer survival outcomes in patients with high level of BNIP3L, although the difference was not statistically significant. This trend may have clinical relevance and warrants further investigation in larger, long-term prospective cohorts to validate the prognostic value of BNIP3L. Furthermore, multivariable analysis confirmed that serum BNIP3L level is an independent prognostic factor for overall survival, with higher levels significantly increasing the risk of mortality (HR = 1.004, 95% CI: 1.001–1.007, $P = 0.021$). This finding is consistent with the report that BNIP3L deficiency significantly delays the progression of pancreatic cancer.[37] In glioblastoma, mitochondrial-localized BNIP3L has been shown to have a pro-tumorigenic effect, promoting tumor cell survival or adaptation.[8]

Of course, this research also has some potential limitations. Firstly, the sample size is not sufficient. Increasing the sample size would make our data more convincing and reliable. It could also help effectively observe changes in the survival rate of patients with different serum BNIP3L levels over time. Secondly, although the study cohort included

patients at different disease stages and under various treatment regimens, the limited sample size within each subgroup precluded statistically robust stratified analyses by specific treatment protocols or disease duration. Additionally, due to the urgent nature of MM symptoms, many patients had already received symptomatic medications from local clinics or pharmacies before being referred to our hospital. This made it difficult to assemble a cohort consisting entirely of untreated, newly diagnosed patients. Future studies with larger prospective cohorts are warranted to overcome this limitation. Thirdly, our study cohort lacked patients with monoclonal gammopathy of undetermined significance (MGUS) or smoldering multiple myeloma (SMM), which limits the exploration of BNIP3L's potential value for early detection. Retrospective or prospective validation of BNIP3L in cohorts of MGUS and SMM patients will be a crucial direction for our future research. Furthermore, it is essential to compare serum BNIP3L levels in MM patients before and after treatment to better understand its role in assessing treatment efficacy. Finally, a longer follow-up period would allow for a more accurate assessment of long-term survival.

Conclusion

Our investigation revealed that serum levels of BNIP3L were significantly elevated in MM patients compared to healthy individuals. This increased expression may be associated with EMI. FISH-defined high-risk cytogenetic abnormalities were associated with significantly elevated serum BNIP3L levels, further correlating this marker with disease aggressiveness. Furthermore, a significant reduction in BNIP3L levels was observed in patients receiving ASCT compared to non-transplanted patients. Additionally, patients who achieved VGPR had significantly lower levels than those achieving only PR. Through survival analysis and ROC curve evaluation, we demonstrated that elevated serum BNIP3L levels are associated with poorer survival. In summary, serum BNIP3L level is a biomarker associated with disease status, genetic risk, and poor prognosis. Furthermore, it dynamically reflects therapeutic efficacy and the depth of remission. Based on these findings, BNIP3L represents a potential dual-purpose biomarker for monitoring progression and predicting outcomes in MM. Nevertheless, definitive confirmation through larger-scale, longitudinal research is essential to validate its application and investigate the mechanistic basis.

Abbreviations

BNIP3L, Bcl-2/adenovirus E1B 19 kDa-interacting protein 3-like; MM, multiple myeloma; Hb, hemoglobin; Plt, platelet; Alb, albumin; Cr, creatinine;UA,uric acid; BUN, blood urea nitrogen; CrCl, creatine clearance; LDH, lactate dehydrogenase; Ca, calcium; β_2-MG, β_2-microglobulin; ESR, erythrocyte sedimentation rate; FLC, free light chain; DS, Durie salmon staging system; ISS, international staging system; R-ISS, revised international staging system; OLs, osteolytic Lesions; EMI, extramedullary infiltration; VGPR, very good partial response; PR, partial response; PD, progressive disease; CI, confidence interval; HR, hazard ratio; OS, overall survival; ROC, Receiver operating characteristic; AUC, Area Under the Curve.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

This study was conducted in strict accordance with the Declaration of Helsinki, and this research program was approved by The Ethics Committee of First Affiliated Hospital of Guangxi Medical University (NO: 2023-E160-01). All participants signed informed consent forms.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interest.

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