Int J Clin Exp Med 2020;13(12):9802-9809
www.ijcem.com /ISSN:1940-5901/IJCEM0117386

Original Article

Study on relationship between serum total light chain κ/λ ratio and typology of multiple myeloma, and the expression of Survivin and Bcl-2 in patients with multiple myeloma

Qiaolin Chen, Xiaofang Wei, Youfan Feng, Qike Zhang

Department of Hematology, Gansu Provincial Hospital, Lanzhou, Gansu Province, China

Received July 1, 2020; Accepted July 29, 2020; Epub December 15, 2020; Published December 30, 2020

Abstract: Objective: To investigate the correlation between total light chain κ/λ ratio (sTLC-κ/λ) in serum and disease types, as well as the levels of Survivin and Bcl-2 in multiple myeloma (MM). Methods: A total of 80 patients confirmed with MM were included in this study and assigned to the MM group. Another 30 patients without plasmacyte proliferative disorders were enrolled in control group in the same period. MM patients were stratified into subgroups; IgGκ MM (n=23), IgGλ MM (n=25), IgAκ MM (n=14) and IgAλ MM (n=18). sTLC-κ/λ was calculated through an automatic protein analyzer and compared among the different groups. The levels of Survivin and Bcl-2 were detected by immunohistochemistry staining and Real-time PCR, and were compared between the MM group and control group. The correlation between sTLC-κ/λ, and disease types and the levels of Survivin and Bcl-2 were performed by Pearson's correlation analysis. Results: Compared with control group, the significant differences in sTLC-κ/λ were found in the MM group (P<0.001). In the IgGκ MM group sTLC-κ/λ was higher than that in the IgAκ MM group, while sTLC-κ/λ in the IgGλ MM group was lower than that in the IgAλ MM group. The positive ratio and levels of Survivin and Bcl-2 in the MM group were significantly higher than those in control group (all P<0.001). sTLC-κ/λ was positively correlated with IgG level in the IgGκ MM group, IgA level in the IgAκ MM group, and the levels of Survivin and Bcl-2 (all P<0.05); and sTLC-κ/λ was negatively correlated with IgG level in the IgGλ MM group and IgA level in the IgAλ MM group (all P<0.05). Conclusion: In MM, sTLC-κ/λ was related with disease type, and the levels of Survivin and Bcl-2 were up-regulated, and sTLC-κ/λ was positively-related with the levels of Survivin and Bcl-2, suggesting that sTLC-κ/λ is a promising biomarker for diagnosis and prognosis monitoring of IgG type and IgA type MM.

Keywords: Multiple myeloma, sTLC-κ/λ, Survivin, Bcl-2

Introduction

Multiple myeloma (MM) is a malignant plasmatic cell disorder and it is characterized by the malignant proliferation of a single clone of plasma cells in the bone marrow, leading to monoclonal protein presenting in the serum and the dysfunction of multiple organs [1, 2]. In recent years, the strategies of treatment that target both the bone marrow microenvironment and MM cells can remarkably prolong overall survival and improve prognosis [3]. It is reported that MM is a heterogeneous disorder and the prognosis of patients can be very different [4]. MM seriously threatens the life quality and physical and psychological health of patients. Therefore, the identification of effective molecular markers that could be help with the diagnosis of MM and predict prognosis would be of significant benefit to patients with MM [5].

Survivin is a protein that is considered an inhibitor of apoptosis and it plays a key role in the process of cell division targeting different components of the mitotic spindle [6]. Some studies have reported that compared with normal bone marrow cells from healthy controls, the expression of Survivin was obviously higher in primary myeloma cells and other myeloma cell lines from patients with MM [7, 8]. Other studies have shown that increased Survivin level was correlated with poor prognosis, disease

progression, and multidrug resistance in patients who suffered from MM [9]. Bcl-2 is another anti-apoptotic protein, and it has been reported that different kinds of tumor cells resist apoptotic cell death by over-expressing Bcl-2 protein [10]. It also was shown that Bcl-2 was responsible for drug resistance and metastatic events in patients with MM [11]. As we can see, Survivin and Bcl-2 play important roles in the development of MM.

In addition, plasma cells and B cells can secrete immunoglobulin (Ig), which mainly consists of a light chain and a heavy chain [12]. The light chain is separated into lambda (λ) and Kappa (κ) light chains. The serum total light chain (sTLC) is composed of the light chain as part of the intact Ig and the free light chain in serum. It has been reported that there are a great many sTLCs in MM patients and they could not be completely metabolized by the kidney in the body, leading to greater than the normal range of serum total light chain κ/λ ratios (sTLC-κ/λ) [13]. Previous studies have reported that in different lymphoma types, sTLC-κ/λ varied considerably, ranging from 0 to 36% [14]. Moreover, some studies have reported that abnormal sTLC-κ/λ was associated with poor prognosis in patients with mantle cell lymphoma or chronic lymphocytic leukemia [15, 16]. However, few reports have studied the relationship of sTLC-κ/λ with disease types in MM patients and the expression levels of Survivin and Bcl-2. Therefore, in this study, we examined the sTLC-κ/λ ratio in patients with different types of MM and the expression levels of Survivin and Bcl-2 in MM patients, with the aim to tentatively confirm whether there were correlations between sTLC-κ/λ with disease types, and the levels of Survivin and Bcl-2. This study may bring insights into the diagnosis and prognostic prediction of MM.

Materials and methods

General data

A total of 80 MM patients who were admitted to our hospital from January 2018 to December 2019 were selected as the subjects in the study. The patients were included if they were over 18 years old, met the diagnostic criteria of multiple myeloma by the method of bone marrow biopsy, and provided written informed consents signed by them or their families. The patients were excluded if they had renal disease, infective diseases within six months of study, malignancy, autoimmune diseases and a history of organ transplantation surgery and previous chemotherapy, or underwent the usage of nephrotoxic drugs within six months, or the long-term usage of glucocorticoids. The patients who had incomplete clinical medical records or were unable to follow the protocol were also excluded. The concurrent patients without plasmacyte proliferative disorders were included in control group (n=30 cases were selected in the control group). All the patients in this study provided written informed consent and the protocols of this study were approved by the Hospital Ethics Committee.

Detection of total serum light chain κ/λ ratio (sTLC-κ/λ)

The sTLC-κ/λ of patients was compared among different groups. At 12 h after fasting, 5 mL venous blood was drawn from the elbow vein of each patient, and then kept in an EDTA anticoagulant tube. The plasma was separated by centrifuging at 3500 r/min for 10 min, and stored at -20°C. The levels of IgG, IgA, light chain κ and light chain λ were detected by automatic protein analyser (BN-II System type, Siemens AG), strictly following the instructions on these kits. Finally sTLC-κ/λ was calculated.

Detection of Survivin and Bcl-2 protein levels by immunohistochemistry staining

Paraffin embedded bone marrow biopsy sections in the MM group and control group were de-waxed and rehydrated through gradients if ethanol into water. Antigen repair was conducted using citrate antigen retrieval solution. In order to eliminate the endogenous peroxidase activity, these sections were incubated with 3% H_2O_2 for 8 min at room temperature. Then 10% normal goat serum was used as blocking reagent and the sections were incubated at room temperature for 30 min. Next, mouse anti-human Survivin/Bcl-2 monoclonal antibody (Santa Company, USA) was added and kept at 4°C in a wet box overnight. After washing with PBS, HRP labeled anti-mouse IgG antibody was added and kept at 37°C in a wet box for 30 min. DAB was applied to show color. Before mounting, these sections were re-stained with hematoxylin. A light microscope was applied to count the color in 200 cells. The positive cells dis-

Table 1. Comparison of basic information of patients among groups

Variable		IgGκ MM group	IgGλ MM group	IgAκ MM group	IgAλ MM group	F/χ^2	P
Case (n)		23	25	14	18	-	-
Male/Female		18/5	19/6	10/4	13/5	0.313	0.956
Age (years)		63.42±4.71	64.13±5.26	63.83±5.01	64.34±4.32	0.139	0.936
Hypertension (n)		5	9	3	6	1.744	0.627
Diabetes (n)		6	8	5	5	0.475	0.924
Hyperlipidemia (n)		7	5	4	5	0.773	0.856
BMI (kg/m^2)		21.36±0.53	21.62±0.84	21.73±0.91	21.51±0.72	1.099	0.355
ISS stage	I	3	4	2	3	0.585	0.996
	II	5	7	3	4		
	III	15	14	9	11		

Note: MM: Multiple myeloma; BMI: Body mass index; ISS: International staging system.

played brownish yellow granules on the surface and cytoplasm of the cells.

Detection of Survivin and Bcl-2 mRNA levels by Real-time PCR

The mononuclear cells were separated from bone marrow in each group by the method of density gradient centrifugation. Trizol reagent (Invitrogen, USA) was used for extracting total RNA. Then, total RNA was synthesized into cDNA through reverse transcription polymerase chain reaction. The primer and probe sequences of GAPDH, Survivin and Bcl-2 were as follows: The GAPDH forward primer was 5'-TGCAGCGTACTCCCCACAT-3' and reverse primer was 5'-TCCATGACAACTTTGGTATCGTG-3'. The Survivin forward primer was 5'-CCTCTGGTGCCACTTTCAAG-3' and reverse primer was 5'-CCAGATGACGACCCCATAGA-3'. The Bcl-2 forward primer was 5'-ACACTGTTAAGCATGTGCCG-3' and reverse primer was 5'-CCAGCTCATCTCACCTCACA-3'. According to the instructions of PCR kits (Thermo Fisher Scientific, USA), Real-time PCR was performed. The reaction system 20 uL included cDNA 2 μL, forward and reverse primers of 0.8 μL each, SYBR Premix Ex TaqTM II (2*) 10 μL, ROX Reference Dye (50*) 0.6 μL, and dH_2O 5.8 μL. PCR amplification was performed in an Applied Biosystems 7500 PCR System. The reaction conditions included initial denaturation at 94°C for 30 sec, denaturation at 94°C for 15 sec, renaturation at 58°C for 1 min, extension at 72°C for 1 min, with 35 cycles. The mRNA relative expression levels of Survivin, and Bcl-2 in each group were calculated with the use of the 2-ΔΔCt method. The GAPDH gene expression was used as the internal reference.

Statistical analysis

Data analysis was conducted using SPSS software, version 21.0. The quantitative data were presented as mean ± standard deviation (Mean ± SD). Comparison between two groups was performed using the student's t test, whereas comparisons among more than two groups were performed using one-way analysis of variance (ANOVA). The correlation of sTLC-κ/λ with the levels of IgG, IgA, Survivin and Bcl-2 were evaluated using Pearson correlation analysis. By contrast, the count data were expressed as percentages or rates, and the chi-square test was used to compare the differences between groups. $P<0.05$ indicated statistically significant differences.

Results

Comparison of basic information among groups

Among the 80 MM patients, there are 48 patients with IgG MM (23 IgGκ MM and 25 IgGλ MM) and 32 patients with IgA MM (14 IgAκ MM and 18 IgAλ MM). Sex ratio, age, ISS stage and DS stage of patients were generally well-balanced among the different groups ($P>0.05$). Data are shown in **Table 1**, and they are comparable.

Comparison of total serum light chain κ/λ ratio (sTLC-κ/λ) among the groups

As shown in **Table 2**, significant differences for sTLC-κ/λ were found in the IgGκ MM group, IgGλ MM group, IgAκ MM group and IgAλ MM group, compared to those in the control group

Table 2. Comparison of sTLC-κ/λ among different groups

Group	Cases (n)	sTLC-κ/λ
Control group	30	1.68±0.42
IgGκ MM	23	95.71±38.57
IgGλ MM	25	0.41±0.06
IgAκ MM	14	38.62±16.55
IgAλ MM	18	0.93±0.28
F		115.300
P		<0.001

Note: MM: Multiple myeloma; sTLC-κ/λ: serum total light chain κ/λ ratio.

Table 3. Correlation coefficients between sTLC-κ/λ and types of multiple myeloma

Types	Correlation coefficient (r)	P
IgGκ MM	0.659	<0.001
IgGλ MM	-0.611	0.014
IgAκ MM	0.596	0.028
IgAλ MM	-0.723	0.005

(all P<0.001). sTLC-κ/λ in the IgGκ MM group was significantly higher than that in the IgAκ MM group, while sTLC-κ/λ in the IgGλ MM group was significantly lower than that in the IgAλ MM group (all P<0.001).

Correlatios between sTLC-κ/λ and types of multiple myeloma

According to Pearson correlation analysis, sTLC-κ/λ was positively correlated with IgG level in the IgGκ MM group and IgA level in the IgAκ MM group. sTLC-κ/λ was negatively correlated with IgG level in the IgGλ MM group and IgA level in the IgAλ MM group. This suggests that there was a certain correlation between sTLC-κ/λ *and types of* Multiple myeloma, as seen in **Table 3**.

Comparison of Survivin level between the MM group and control group

The results of immunohistochemical staining showed that the positive ratio of Survivin in bone marrow mononuclear cells in the MM group was significantly higher than that in control group (5.00% vs 30.00%, χ^2=6.580, P< 0.001), as seen in **Figure 1**. The qPCR results showed that the level of Survivin mRNA in the MM group was significantly higher than that in the control group (P<0.001), as seen in **Table 4**.

Comparison of Bcl-2 level between the MM group and control group

The results of immunohistochemical staining showed that the positive ratio of Bcl-2 in bone marrow mononuclear cells in the MM group was obviously higher than that in the control group (4.00% vs 35.00%, χ^2=7.152, P<0.001), as seen in **Figure 2**. The qPCR results showed that the level of Bcl-2 mRNA in the MM group was significantly higher than that in the control group (P<0.001), as seen in **Table 5**.

Correlatios between sTLC-κ/λ and the levels of Survivin and Bcl-2

Pearson correlation analysis revealed that sTLC-κ/λ was positively correlated with the level of Survivin (r=0.642, P=0.016), as well as the level of sTLC-κ/λ and Bcl-2 (r=0.686, P=0.022), as seen in **Table 6**.

Discussion

Multiple myeloma is a type of hematological malignant tumor and the incidence is increasing on a annual basis, due to an ageing population and environmental factors [17]. It is reported that IgG MM is the most common type with accounting for 50%-60% of the total cases and that IgA MM accounts for 15%-20% of the total cases [18]. In this study, among the 80 MM patients, there were 48 patients with IgG MM and 32 patients with IgA MM. The percentage of IgG MM and IgA MM in the total cases was basically similar with the results of current epidemiological investigations [19]. In recent years, significant progress has been made in diagnostic methods, molecular mechanisms and prognostication in MM. Serum monoclonal proteins such as serum-free light chain assays, and serum factors have been used to predict the prognosis of MM patients. From this, it is likely that more targeted and risk-adapted treatment methods may be used to treat MM patients at different stages guided by the potential biomarkers.

Survivin has been reported to be associated with pathological parameters and adverse clinical responses in various hematopoietic tumors [20]. Romagnoli et al reported that Survivin expression detected by western blot was related with clinical stages [8]. In this study, the positive expression rate of Survivin in MM was

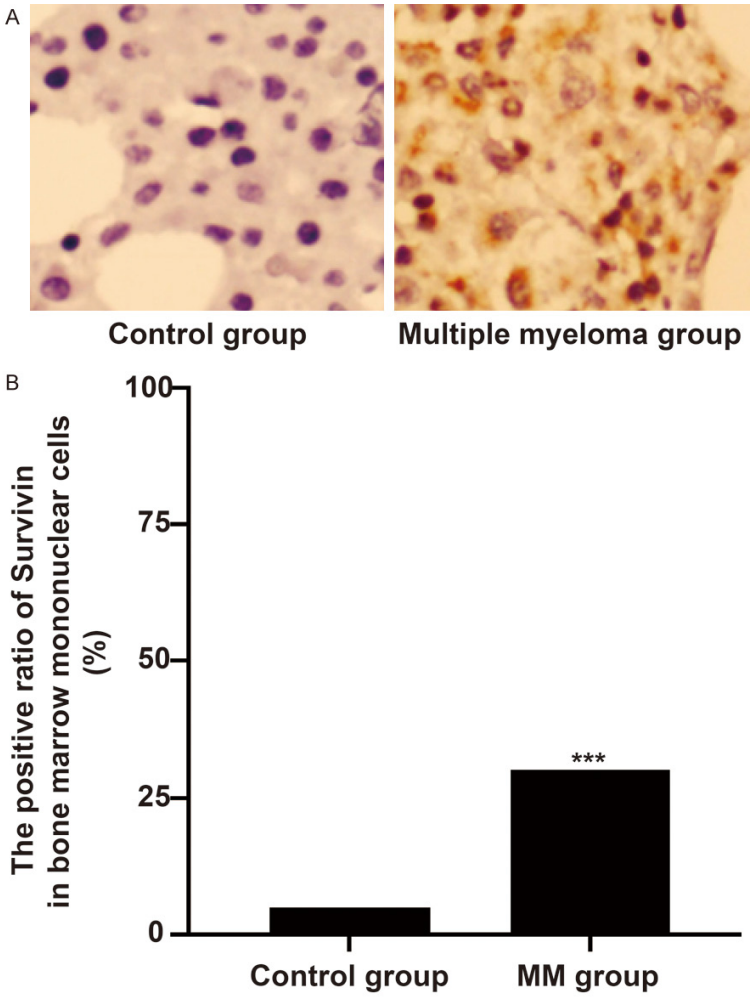

Figure 1. Comparison of the positive ratio of Survivin in bone marrow mononuclear cells between two groups. A. The expression of Survivin in bone marrow plasmacyte by immumohistochemical staining. B. Quantitative analysis of the positive ratio of Survivin. Compared with control group, ***P<0.001. Note: MM: Multiple myeloma.

Table 4. Comparison of Survivin mRNA level between two groups

Groups	Cases	Survivin mRNA
Control group	30	0.18±0.04
MM group	80	0.47±0.06
T		24.480
P		<0.001

Note: MM: Multiple myeloma.

30%, which was significantly higher than that in control group. To date, the Survivin expression in MM using immunohistochemical staining has not been investigated. This study also showed that Survivin expression measured by qPCR in MM patients was obviously higher than that in control group. In addition, it was reported that overexpression of Bcl-2 indicated sustained malignant proliferation of MM tumor cells [21]. Another study showed that the positive expression rate of Bcl-2 in MM was about 43%-75% [22]. This study reported that the Bcl-2 positive expression rate was 35%, which was higher than that in control group. qPCR results showed that the level of Bcl-2 in MM patients was higher than that in control group. These findings indicated that Survivin and Bcl-2 expression could reflect tumor conditions in MM and are associated with development of MM.

Serum light chain has been considered as one of the sensitive indicators for the assessment of clonal plasma cells. The level of normal serum κ light chain is approximately two times of that of λ light chain. Also, sTLC-κ/λ is not affected by renal function and blood volume, which can somewhat accurately reflect the expression levels of polyclonal immune globulin. It is demonstrated that sTLC-κ/λ plays an important role in the diagnosis of MM. The results of this study showed that compared with that in control group, a significant difference in sTLC-κ/λ was found in the IgGκ MM group, IgGλ MM group, IgAκ MM group and IgAλ MM group. Compared with that in the IgAκ MM group, sTLC-κ/λ in the IgGκ MM group was remarkably higher. Compared with that in the IgAλ MM group, sTLC-κ/λ in the IgGλ MM group was obviously lower. Moreover, Pearson correlation analysis results showed that sTLC-κ/λ was closely associated with IgG MM and IgA MM. These findings are basically consistent with results reported by Durie et al [23]. This indicates that sTLC-κ/λ in the diagnosis of IgG MM and IgA MM has important clinical value and the increased level of serum monoclonal

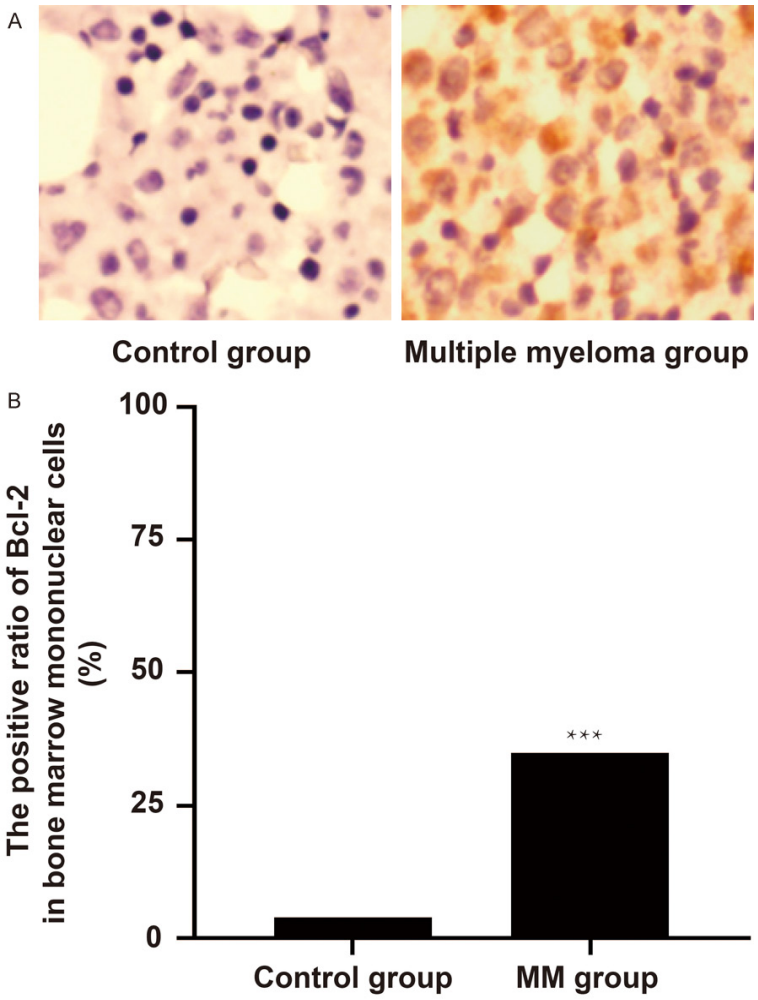

Figure 2. Comparison of the positive ratio Bcl-2 in bone marrow mononuclear cells between two groups. A. The expression of Bcl-2 in bone marrow plasmacyte by immumohistochemical staining. B. Quantitative analysis of the positive ratio of Bcl-2. Compared with control group, ***P<0.001. Note: MM: Multiple myeloma.

Table 5. Comparison of Bcl-2 mRNA level between two groups

Groups	Cases	Bcl-2 mRNA
Control group	30	0.11±0.03
MM group	80	0.58±0.09
T		27.960
P		<0.001

Table 6. Correlation coefficients between sTLC-κ/λ and the levels of Survivin and Bcl-2

Variable	Correlation coefficient (r)	P
Survivin	0.642	0.016
Bcl-2	0.686	0.022

protein and suppressive degree of normal serum immunoglobulins in IgG MM are more than those in IgA MM. It also is demonstrated that the serum levels of IgG and IgA are the main cause of sTLC-κ/λ imbalance in patients with IgG MM and IgA MM.

For the past few years, sTLC-κ/λ in MM has been applied to evaluate the therapeutic treatment effect by many scholars [24]. This study analyzed the correlation of sTLC-κ/λ with the expression levels of Survivin and Bcl-2 in MM and the results showed that sTLC-κ/λ was positively correlated with the levels of Survivin and Bcl-2, which indicated that sTLC-κ/λ could provide a clinical reference for the evaluation of prognosis in patients with MM. The relationship of sTLC-κ/λ with the expression levels of Survivin and Bcl-2 has not been reported in previous studies. Some studies showed that the expression of Survivin was not related with clinical stage in patients with newly diagnosed multiple myeloma [25]. Guo et al [26] reported that Bcl-2 was related with poor prognosis including progression-free survival and shorter overall survival in patients with solitary bone plasmacytoma. Renner et al [27] reported that Bcl-2 was not associated with various quantitative variables such as age, C-reactive protein and β2-microglobulin in patients with MM.

In conclusion, sTLC-κ/λ has great significance in IgG MM and IgA MM, and patients with MM have elevated Survivin and Bcl-2 concentration. The sTLC-κ/λ ratio is closely associated with IgG MM, IgA MM and the levels of Survivin and Bcl-2. However, the specific mechanism of sTLC-κ/λ in the pathogenesis of MM remains unclear, requiring further experimental investigation.

Acknowledgements

This work was supported by Grants from the Establishment and Application of Collaboration Patterns for Multi-disciplinary Treatment of Multiple Myeloma (17GSSY2-2).

Disclosure of conflict of interest

None.

Address correspondence to: Qike Zhang, Department of Hematology, Gansu Provincial Hospital, No. 204 Donggang West Road, Chengguan District, Lanzhou 730030, Gansu Province, China. Tel: +86-0931-8281203; Fax: +86-0931-8281203; E-mail: zhangqike_gshos20@163.com

References

[1] Brigle K and Rogers B. Pathobiology and diagnosis of multiple myeloma. Semin Oncol Nurs 2017; 33: 225-236.

[2] Rajkumar SV. Multiple myeloma: every year a new standard? Hematol Oncol 2019; 37 Suppl 1: 62-65.

[3] Pawlyn C and Davies FE. Toward personalized treatment in multiple myeloma based on molecular characteristics. Blood 2019; 133: 660-675.

[4] Joshua DE, Bryant C, Dix C, Gibson J and Ho J. Biology and therapy of multiple myeloma. Med J Aust 2019; 210: 375-380.

[5] Levin A, Hari P and Dhakal B. Novel biomarkers in multiple myeloma. Transl Res 2018; 201: 49-59.

[6] Ding JH, Yuan LY and Chen GA. Aspirin enhances the cytotoxic activity of bortezomib against myeloma cells via suppression of Bcl-2, Survivin and phosphorylation of AKT. Oncol Lett 2017; 13: 647-654.

[7] Romagnoli M, Seveno C, Wuilleme-Toumi S, Amiot M, Bataille R, Minvielle S and Barille-Nion S. The imbalance between Survivin and Bim mediates tumour growth and correlates with poor survival in patients with multiple myeloma. Br J Haematol 2009; 145: 180-189.

[8] Romagnoli M, Trichet V, David C, Clement M, Moreau P, Bataille R and Barille-Nion S. Significant impact of Survivin on myeloma cell growth. Leukemia 2007; 21: 1070-1078.

[9] Tsubaki M, Satou T, Itoh T, Imano M, Komai M, Nishinobo M, Yamashita M, Yanae M, Yamazoe Y and Nishida S. Overexpression of MDR1 and Survivin, and decreased Bim expression mediate multidrug-resistance in multiple myeloma cells. Leuk Res 2012; 36: 1315-1322.

[10] Khan N and Kahl B. Targeting BCL-2 in hematologic malignancies. Target Oncol 2018; 13: 257-267.

[11] Ailawadhi S, Miecznikowski J, Gaile DP, Wang D, Sher T, Mulligan G, Bryant B, Wilding GE, Mashtare T, Stein L, Masood A, Neuwirth R, Lee KP and Chanan-Khan A. Bortezomib mitigates adverse prognosis conferred by Bcl-2 overexpression in patients with relapsed/refractory multiple myeloma. Leuk Lymphoma 2012; 53: 1174-1182.

[12] Yadav P, Merz M, Mai EK, Forsti A, Jauch A, Goldschmidt H and Hemminki K. Cytogenetic aberrations in multiple myeloma are associated with shifts in serum immunoglobulin isotypes distribution and levels. Haematologica 2018; 103: e162-e164.

[13] Zhu AY, Zhu FB, Wang FC, Zhang LJ, Ma Y and Hu JG. Analysis of relationship between serum total light chain kappa/lambda ratio and proportion of bone marrow plasma cells in patients with IgG type and IgA type multiple myeloma. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2017; 25: 1454-1459.

[14] Martin W, Abraham R, Shanafelt T, Clark RJ, Bone N, Geyer SM, Katzmann JA, Bradwell A, Kay NE and Witzig TE. Serum-free light chain-a new biomarker for patients with B-cell non-Hodgkin lymphoma and chronic lymphocytic leukemia. Transl Res 2007; 149: 231-235.

[15] Furtado M, Shah N, Levoguer A, Harding S and Rule S. Abnormal serum free light chain ratio predicts poor overall survival in mantle cell lymphoma. Br J Haematol 2013; 160: 63-69.

[16] Pratt G, Harding S, Holder R, Fegan C, Pepper C, Oscier D, Gardiner A, Bradwell AR and Mead G. Abnormal serum free light chain ratios are associated with poor survival and may reflect biological subgroups in patients with chronic lymphocytic leukaemia. Br J Haematol 2009; 144: 217-222.

[17] Gerecke C, Fuhrmann S, Strifler S, Schmidt-Hieber M, Einsele H and Knop S. The diagnosis and treatment of multiple myeloma. Dtsch Arztebl Int 2016; 113: 470-476.

[18] Rome S, Noonan K, Bertolotti P, Tariman JD and Miceli T. Bone health, pain, and mobility: evidence-based recommendations for patients with multiple myeloma. Clin J Oncol Nurs 2017; 21: 47-59.

[19] Bertolotti P, Pierre A, Rome S and Faiman B. Evidence-based guidelines for preventing and managing side effects of multiple myeloma. Semin Oncol Nurs 2017; 33: 332-347.

[20] Lyu H, Huang J, He Z and Liu B. Epigenetic mechanism of Survivin dysregulation in human cancer. Sci China Life Sci 2018; 61: 808-814.

[21] Touzeau C, Maciag P, Amiot M and Moreau P. Targeting Bcl-2 for the treatment of multiple myeloma. Leukemia 2018; 32: 1899-1907.

[22] Sangfelt O, Osterborg A, Grander D, Anderbring E, Ost A, Mellstedt H and Einhorn S. Response

to interferon therapy in patients with multiple myeloma correlates with expression of the Bcl-2 oncoprotein. Int J Cancer 1995; 63: 190-192.

[23] Durie BG, Harousseau JL, Miguel JS, Bladé J, Barlogie B, Anderson K, Gertz M, Dimopoulos M, Westin J, Sonneveld P, Ludwig H, Gahrton G, Beksac M, Crowley J, Belch A, Boccadaro M, Cavo M, Turesson I, Joshua D, Vesole D, Kyle R, Alexanian R, Tricot G, Attal M, Merlini G, Powles R, Richardson P, Shimizu K, Tosi P, Morgan G and Rajkumar SV; International Myeloma Working Group. International uniform response criteria for multiple myeloma. Leukemia 2006; 20: 1467-1473.

[24] Dejoie T, Corre J, Caillon H, Moreau P, Attal M and Loiseau HA. Responses in multiple myeloma should be assigned according to serum, not urine, free light chain measurements. Leukemia 2019; 33: 313-318.

[25] Zeng W, Meng F, Liu Z, Mao X, Luo L, Zheng M, Qin S, Liu W, Zhou J, Sun H and Huang L. Bortezomib-based chemotherapy regimens can improve response in newly diagnosed multiple myeloma patients with bcl-2 and Survivin overexpression. Int J Clin Exp Pathol 2014; 7: 4239-4246.

[26] Guo S, Zhi Y, Yang H, Yu Y, Wang Y, Zhang J, Wang G, Zhang L, Sun B and Zhang Y. Bcl-2 expression is associated with poor prognosis of solitary plasmacytoma of bone. Ann Hematol 2014; 93: 471-477.

[27] Renner S, Weisz J, Krajewski S, Krajewska M, Reed JC and Lichtenstein A. Expression of BAX in plasma cell dyscrasias. Clin Cancer Res 2000; 6: 2371-2380.