RESEARCH ARTICLE

The Correlation Between TP53 Expression and Ki-67 Proliferation with Bartl Malignancy Degree of Plasma Cell Neoplasm

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Abstract

BACKGROUND: Plasma cell neoplasm (PCN) is a neoplastic plasma cell proliferation which includes solitary bone plasmacytoma (SBP), extramedullary plasmacytoma (EMP) and multiple myeloma (MM). Bartl classifies the degrees of PCN as low, intermediate and high. The aim of this study is to find the correlation between tumor suppressor gene p53 (TP53) expression and Ki-67 proliferation with Bartl malignancy degree of PCN. Therefore earlier PCN diagnostic method to prevent the development of PCN into MM can be found.

METHODS: Thirty-two PCN cases were classified into three groups based on Bartl's degrees of malignancy. TP53 and Ki-67 immunohistochemical staining were performed on samples and the percentage of positivity was evaluated.

RESULTS: The Bartl's low degree of malignancy was found in 10 MM cases (31.2%), intermediate degree in 5 SBP cases (15.6%) and high in 2 SBP and EMP cases (6.2%). TP53 expression was obtainable at 4% of low, 16% of intermediate and 10% of high degree. There was a significant difference between TP53 expression in low and intermediate degree (p=0.004). Mean proliferation index of Ki-67 was 57% in low, 44.6% in intermediate, and 32.6% in high degree. There was no significant difference of Ki-67 proliferation indexes among the group (p=0.339).

CONCLUSION: Increasing expression TP53 was in accord with Bartl's degrees of malignancy, especially in low and intermediate degree, but there was no significant difference between Ki-67 proliferation index and Bartl's degrees of malignancy.

KEYWORDS: plasmacytoma, myeloma, TP53, Ki-67, Bartl classification

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Introduction

World Health Organization (WHO) classifies plasmacytoma into a neoplastic plasma cell without any infiltration to the bone marrow, including Solitary Bone Plasmacytoma (SBP), Extramedullary Plasmacytoma (EMP), and abnormal plasma cells (myeloma cells) build up in the bone marrow including Multiple Myeloma (MM).(1-3) More than 80% EMP developed in upper respiratory tract which has many lymphoid tissues and 17-33% grow into MM.(4-6)

MM is classified into six cell types due to its size, cytoplasmic structure and the nucleus configuration. The classification is also associated with the prognosis degrees, which can be divided into low (40 months survival), medium (20 months survival) and high malignancy degree (8 months survival).(7)

SBP can develop into MM in the average of 21 months.(1,3) Around 65-84% SBP develops into MM in 10 years, and 65-100% of them grows in 15 years. EMP has 100% of 5-year-survival-rate, and 70% of 10-year-survival-rate. Those who developed into MM had 70% of 5-year-

survival-rate, compare to 33% patients with SBP.(8,9) MM prognosis was worse with the mean of 3-4 years, and most of them were less than six months. Plasma cell neoplasm (PCN) prognosis was affected by age, tumor size, and location.(1,3)

The development of myeloma comes from premalignant stage known as monoclonal gammopathy of unknown significance (MGUS) and become MM in the suitable microenvironment in bone, soft tissues and bone marrow. Until now, the development from MGUS to MM cannot determine as predicting factor.(10) Genetic changes, such as oncogene activation and tumor suppressor gene mutation, clinically affect the aggressivity of MM. Tumor suppressor gene p53 (TP53) has a role in genomic stability and cell cycle.(5) Mutation of this protein in locus 17p13 was found in 5-10% myeloma cases (11), suggested that p53 protein could be used as a clinical prognostic marker (6).

The correlation between cluster of differentiation (CD)56 and TP53 to Mindbomb E3 ubiquitin-protein ligase 1 (MIB-1), in intramedullary and extramedullary lesion progression in MM formation was found, but CD56 expression difference between EMP and MM was not. TP53 expression and proliferation activity, the MIB-1, were correlated with tumor aggressivity.(12) A study showed that microvascular density and Ki-67 staining, as a marker of proliferation activity, were significantly higher in untreated plasmacytoma patients. The expression of Ki-67 was correlated with PCN infiltration.(6,10) It might be the reason why the expression of TP53 and Ki-67 could be used for progressivity and prognosis marker in PCN.(5,6,13-16) Diagnosis and staging of PCN were determined by clinical finding, radiologic (lesion found in soft tissues, bone, or bone marrow), and histopathologic data.

The aim of this study is to find the correlation between TP53 expression and Ki-67 proliferation with Bartl malignancy degree of PCN. Therefore earlier PCN diagnostic method to prevent the development of PCN into MM can be found.

Methods

The study was a retrospective cross-sectional using all cases of intramedullary and extramedullary plasma cell tumor, especially in bone or soft tissues which were recorded between 2011-2014 in Department of Pathology Anatomy, Faculty of Medicine, University of Indonesia/ dr. Cipto Mangunkusumo Hospital (FKUI/RSCM), Jakarta. Institutional approval (Health Research Ethics Committee Faculty of Medicine Universitas Indonesia, #370/UN2. F1.ETIK/2015) was taken prior to the study. The clinical data was obtained from RSCM medical record. Damaged samples block were excluded. Samples were determined as SBP, EMP or MM based on WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.(3)

Histopathologic Slides Reexamination

The morphological classification using Bartl`s morphological variants was performed. After the overview, 2-10 power fields were chosen from slides. Bartl classifies six histologic types: (1) Marschalko, (2) small cell, (3) cleaved, (4) polymorphous, (5) asynchronous, and (6) blastic. The subtypes were determined based on the cell type that mostly counted in slides and the highest malignancy degree. These six subtypes then would be grouped into low (Marschalko and small cell), medium (cleave, polymorphous and asynchronous), and high degree malignancy (blastic).

TP53 and Ki-67 Immunohistochemical Expression

The sections were cut from paraffin blocks, and the routine immunohistochemical methods were used to detect TP53 and Ki-67 protein. TP53 protein (DO-7) (Leica Biosystem, Newcastle, UK) and Ki-67 primer monoclonal antibody (Biocare Medical, Concord, USA) were used.

Horseradish peroxidase (HRP) labeled TrekAvidin, phosphate-buffered saline (PBS) washing, and diaminobenzidine (DAB) was added to the slides. The slides were stained with Mayer hematoxylin (Biocare Medical, Concord, USA) before sequentially washed with running water, saturated lithium carbonate (5% in aqua dest) and another running water. A series of increasing alcohol concentrations were added to dehydrate the slides, and for clearing, xylol was also added. Finally, slides were covered with Entellan® (Merck Millipore, Wisconsin, USA) and glass cover. Colon adenocarcinoma tissue was used for TP53 positive control, and tonsil tissue was used for Ki-67 positive control. Negative control was prepared using the same procedures but with no primer antibody.

TP53 and Ki-67 Expression

Specimens' digital photos were taken using Leica ICC 50 HD microscope (Leica Microsystems, Wetzlar, Germany), and brown-stained nuclei were counted at about 300 cells using ImageJ® software (NIH Image, Bethesda, USA). The numeric variable was applied with no cut off.

Statistical Analysis

The statistical analysis was performed by SPSS 12 (IBM SPSS, New York, USA). Shapiro-Wilk test showed that the samples were not normally distributed, so the correlation between TP53 and malignancy degree was utilized with Kruskal-Wallis non-parametric test and Mann-Whitney post hoc test, while the correlation between Ki-67 and malignancy degree was performed using unpaired numeric variable hypothesis test, one-way ANOVA, continued with Bonferroni post hoc test. Correlation between TP53, Ki-67, and age was performed using Spearman correlation test, with significance $p<0.05$.

Results

Samples Characteristics

A total of 32 cases (16 men and 16 women) were reevaluated during the study period. In both genders was found that most cases are the low-grade malignancy. There was no correlation between sex and malignancy ($p=0.198$). The cases were distributed in subjects between 21-72 years old, most of them were in their 50s (13 cases). Most malignancy situations were found in bone (15 cases) (Table 1). Five cases were found to have multiple or recurrent lesions, where 4 of them were categorized as SBP and one case as MM. Two cases were found as repetitive with low malignancy degree, 1 case recurrent with medium malignancy degree and 2 cases with high malignancy degree.

Based on malignancy degree (Bartl classification), we found 10 cases (31.3%) of Marschalko subtype, 8 cases (25%) of small subtype, 4 cases (12.5%) of cleaved subtype, 2 cases (6.2%) of polymorphous subtype, 3 cases (9.4%) of asynchronous subtype and 5 cases (15.6%) of blastic subtype (Figure 1).

Other histological images were also found in reevaluation, including 19 “blood lake” cases (59.3%), 11 Dutcher body cases (34.3%) and 3 amyloid cases (9.3%). Diagnosis showed most MM was found in low malignancy degree (10 cases; 31.2%), most SBP found in low malignancy degree (7 cases; 21.8%), and most EMP found in medium and high malignancy degree (2 cases; 6.2%) (Table 1).

TP53 Expression and Ki-67 Proliferation Activity

It was found that 9 cases were negative or no positive stained nuclei TP53 expression. Eight cases showed to have low malignancy degree, which was found in bone and bone marrow. Meanwhile, one case of medium malignancy degree was found in soft tissue.

Shapiro-Wilk test showed that data were not normally distributed. Kruskal-Wallis test showed a significant difference between malignancy degrees ($p<0.05$), while Mann-Whitney post hoc test showed a significant difference between low and medium malignancy degree, but no difference between high and medium ($p=0.386$) or low ($p=0.101$) malignancy degree.

Table 1. The characteristics of the study group from data 2011-2014.

Demographic Data	Malignancy Degree			Total (%)
	Low	Medium	High	
Gender				
Male	11	3	2	16 (50%)
Female	7	6	3	16 (50%)
Age				
21-30	1	0	0	1 (3.1%)
31-40	1	1	0	2 (6.3%)
41-50	2	3	1	6 (18.8%)
51-60	9	2	2	13 (40.6%)
61-70	5	3	1	9 (28.1%)
>70	0	0	1	1 (3.1%)
Location				
Bone Marrow	9	2	0	11 (34.4%)
Bone				
Thoracic	3	1	0	4 (12.5%)
Femur	2	1	2	5 (15.6%)
Humerus	0	3	0	3 (9.4%)
Lumbal	1	0	0	1 (3.1%)
Clavicula	1	0	0	1 (3.1%)
Mandibula	1	0	0	1 (3.1%)
Extramedullary				
Head&Neck	1	1	1	3 (9.4%)
Thorax	0	0	1	1 (3.1%)
Intracranial	0	1	1	2 (6.3%)
Diagnosis				
MM	10	2	1	13 (40.6%)
SBP	7	5	2	14 (43.8%)
EMP	1	2	2	5 (15.6%)
Total	**18 (56.3%)**	**9 (28.1%)**	**5 (15.6%)**	**32 (100%)**

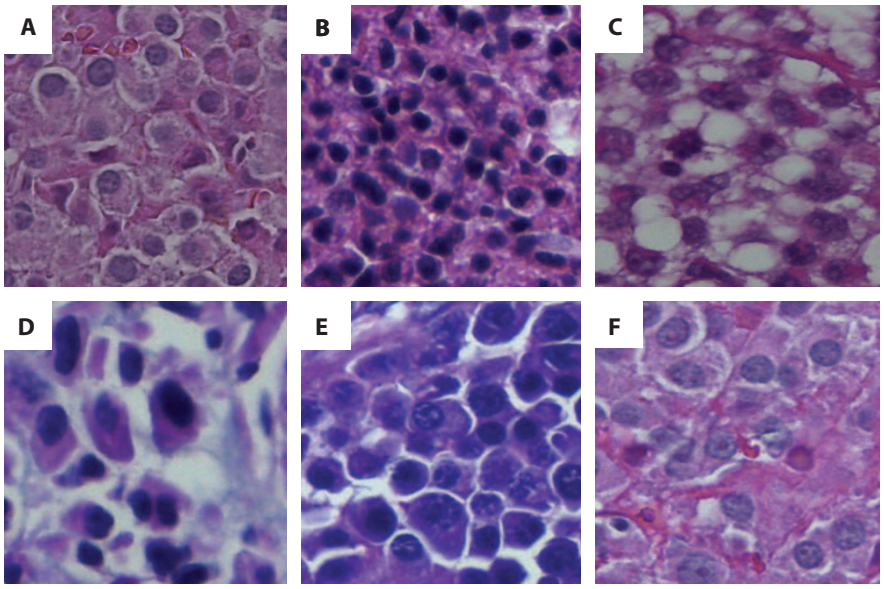

Figure 1. Bartl's PCN classification based on malignancy degree. A: marschalko subtype; B: small cell subtype; C: cleaved subtype; D: polymorphous subtype; E: asynchronous subtype; F: blastic subtype. (Magnification: 400x).

Menawhile, the Shapiro-Wilk test showed a normally distributed data for Ki-67 staining expression. No significant difference was found in this staining.

TP53 Expression and Ki-67 Proliferation Index Determination on SBP and EMP

The differences of TP53 and Ki-67 expression were observed in different malignancy degree for SBP and EMP cases, and were found no differences (p=0.129) (Table 2B).

TP53 Expression and Ki-67 Proliferation Index Determination on PCN Diagnosis

We found high expression of TP53 (median 15%; interval 0-55%), and Ki-67 proliferation index (mean 42.5%) in SBP, and low expression of TP53 (median 9%; interval 0-37%) and Ki-67 proliferation index (mean 30.67%) in MM cases (Table 3). There was no correlation between TP53 expression and Ki-67 expression, as well as no correlation between both to age (p>0.05).

Discussion

The prevalence of hematopoietic neoplasm in the USA recorded 20,000 cases in 2010. In the same year, Indonesia Cancer Registry found 0.43% (107 of 24,781 total neoplasms) cases of hematolymphoid solid tumor. There were no gender differences for hematopoietic neoplasm. PCN was found only 1% from all neoplasm and about 10% from hematopoietic neoplasm, majority in patients' age

Table 2A. Result of TP53 and Ki-67 expression based on to Bartl classification on SBP, EMP and MM.

Malignancy degree	n	TP53			Ki-67	
		Median	Interval (min-max)	*p*	Mean±SD	*p*
Low	18	4	0-29	0.010	57±25.32	0.339
Medium	9	16	0-55		44.67±21.09	
High	5	10	6-42		32.6±5.12	

Table 2B. The difference of TP53 expression based on to Bartl classification on SBP and EMP.

Malignancy degree	n	TP53		
		Median	Interval (min-max)	*p*
Low	8	5	0-29	0.129
Medium	7	16	0-55	
High	4	19.5	6-42	

Table 3. Result of TP53 and Ki-67 expression on PCN.

Diagnosis	n	TP53		Ki-67
		Median (%)	Interval (min-max)	Mean (%)
SBP	14	15	0-55	43
EPM	5	10	0-29	34
MM	13	9	0-37	31

between 55-64 years old.(1-3) PCN are the diseases where the abnormal plasma cells or myeloma cells form tumors in the bone or soft tissue.(4) In this study, PCN cases have 1:1 ratio between men and women and most cases were found in fifth decades of life. Previous study found no prevalence difference between men and women (3), while another study found a 1.8:1 ratio differences (1). Dores, *et al.,* stated a 2:1 ratio in men *vs.* women with SBP, 2.6:1 for EMP, and 1.5:1 for MM.(9) It was also found that predominant men have 6:1 ratio to women.(17)

Our study found MM cases in age 40-67 with median 59 years old, SBP cases in age 21-70 years old with median 52 years old, and EMP cases in age 48-72 years old with median 61 years old. Mean age for SBP and EMP in WHO study was 55 years old with 2:1 ratio of men *vs.*women.(1,3) Meanwhile, other studies found that median age for MM is 65 years old (3,7,18), for SBP is 60 years old and for EMP 55 years old (8). Overall, most cases in our study were found in fifth decades of life, while other studies found their cases in sixth or seventh decades of life. Accordance to WHO, SBP most frequently found in the femur, meanwhile EMP in the head-neck region.(3,18,19) Two EMP cases were found in intracranial, both in lobus frontalis, with medium and high malignancy degrees. Cranial base is the most frequent location for intracranial plasmacytoma.(20)

Clinically, 26 cases in our study showed hemoglobin level between 5.4-15.2 mg/dL. Anemia could happen in PCN, although there were other factors like chronic illness can play a role.(3) Al-Farsi also reported normochromic anemia in 75% of cases, due to increased plasma cell in peripheral blood test.(21) In monoclonality determination, 12 cases from 17 were found as monoclonal. Bence Jones (BJ) Protein test only performed in 4 cases and found one positive case. In urine or serum test was found 99% monoclonality and protein M, and 75% of the cases were positive for BJ protein. Kidney impairment could be induced by hypercalcemia and rarely by amyloidosis.(3) Pratt, who performed a conventional test, only found 40% cases with clonal abnormality, and only 20-30% were found on diagnosis.(22) Due to Durie & Salmon staging, we found only 20% frequency for stage 1, and 60% for stage 3. This happened because of the low level of proliferation in neoplastic plasma cell. Around 13% positive BJ protein was found in SBP cases.(23)

International Myeloma Working Group (IMWG) recommended urine and serum monoclonal protein routine test for any suspect B-cell neoplasm and MM. Any monoclonal finding without PCN signs will be diagnosed as MGUS.(24) Patients with >10% bone marrow plasma cell without any >30 g/L M protein finding in urine/serum will be diagnosed with asymptomatic myeloma or smoldering myeloma, which is biologically similar to MGUS, and have to be strictly followed up due to its fast progression to MM. There was no correlation between surface marker, M component, morphologic classification, and clinical stage in MM and MGUS.(7)

We didn't exclude 11 cases from bone marrow because they have supported clinical data including monoclonality, positive BJ protein, immunohistochemistry $CD38^{+}$ and $CD138^{+}$, $CD19^{-}$ and $CD3^{-}$ as a compared diagnosis for PCN.(3,15,25)

Generally, all neoplastic plasma cell histological images were all similar. However, each malignancy degree has their unique characteristic, such as mild until medium plasma cell dysplasia, increasing cell size, pleomorphism, increasing N:C ratio, having more than one nuclei and mitosis, or even having a giant plasma cell with many nuclei. Basophilic or eosinophilic cytoplasm with flaming peripheral blood smear could have one or more cytoplasm inclusions. Dutcher body could also be seen as nuclear inclusion images.(3,18) Zuo, *et al.,* described "blood cell lake" as one image found in plasmacytoma.(19) Amyloid could be found in 10-15% cases of plasma cell myeloma in bone marrow or bone.(3,18) Due to Bartl classification (26,27), this study showed 19 cases (59.3%) with "blood cell lake" histological image, 11 cases (34.3%) with Dutcher body and 3 cases (9.3%) with amyloid. It is less if compared to Zuo's study where 37.5% "blood cell lake," 12.5% Dutcher body and 18.8% amyloid deposition.

Our population showed 18 cases (56.3%) of low malignancy degree, 9 cases (28.1%) of medium degree and 5 cases (15.6%) of high degree. The most number subtype we found was Marschalko subtype (10 cases), followed by small cell subtype (8 cases), both were low malignancy degree, same as a study by Jayashankar, *et al.,* who found Marschalko as the most frequent subtype.(28) The nucleolus is suggested as the most significant characteristic to determine malignancy degree (29), but irregular cytoplasm image and plasma cell anisocytosis should be noticed as well. Intra-observer variation was 33%, so the best way

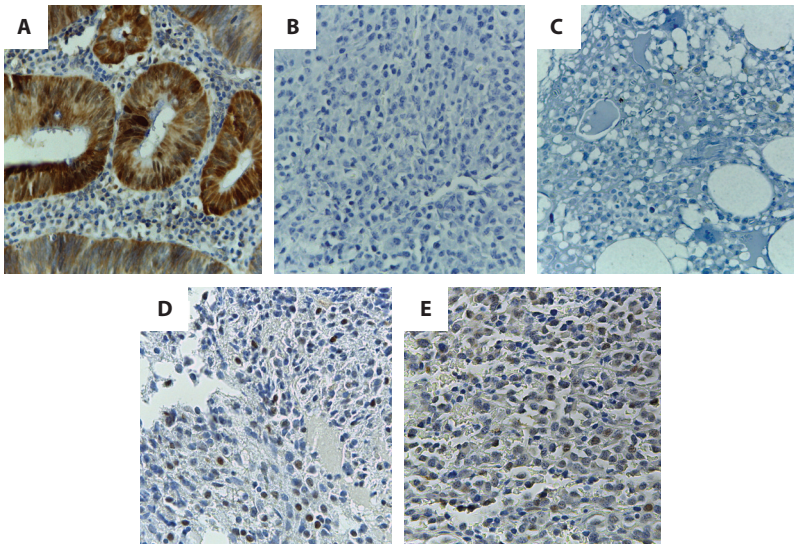

Figure 2. TP53 staining on PCN. A: TP53 positive control, with brown-stained nuclei, considered as positive; B: negative control, no brown staining found, considered as negative and/or 0%; C: low malignancy degree of TP53 staining image (0%); D: medium degree (37%); E: high degree (42%). (Magnification: 100x).

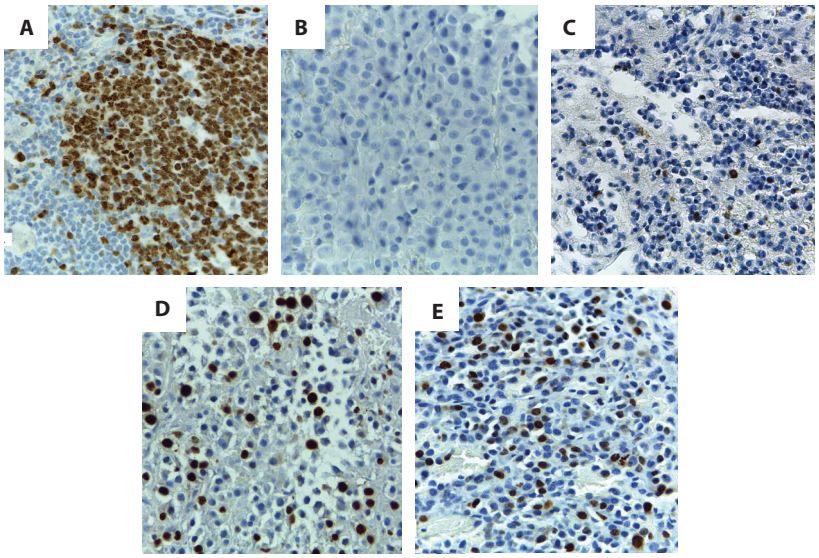

Figure 3. Ki-67 staining on PCN. A: Ki-67 positive control, with brown-stained nuclei, considered as positive; B: negative control, no brown staining found, considered as negative; C: low malignancy degree Ki-67 staining image (10%); D: medium degree (35%); E: high degree (50%). (Magnification: 400x).

to predict this character was to count the plasma cell. Our data showed most cases were in low malignancy degree compare to the medium and high degree, either in MM (10:2:1), SBP (7:5:2) and EMP showed 1:2:2 ratio. It means that we can not use Bartl classification to predict the malignancy progression become MM, since MM was mostly found in low malignancy degree.

We observed the recurrence cases and found 5 cases with recurrent time between 1-22 months. They were 3 with low malignancy degree, 1 with medium degree and 2 with high degree. One SBP case developed into MM with low malignancy degree. Four recurrent cases found in bone, without any progression to MM and have no neoplastic plasma cell in bone marrow. Lorsbach stated that SBP has a higher risk to develop into MM (65-84%) in 10 years, and (65-100%) in 15 years.(8) Another study showed that 60% SBP cases and 20% EMP cases progressed into MM in three years.(30,31) No plasmacytoma patients died or developed into myeloma in about 50 months.(17)

Table 2 showed TP53 and Ki-67 expression in this study. TP53 was significantly different in each malignancy stages (p=0.01). TP53 expression was correlated with

intramedullary and extramedullary lesion development to a higher degree.(12) A lot of p53 mutations in higher level and aggressive MM.(6) IMWG stated that a high number of neoplastic plasma cell in peripheral blood smear and its proliferation activity in bone marrow could be a risk factor for immediately MM development. Until now, there were no fixed methods and sensitive cytometer devices used globally.

A study claimed that β2-microglobulin and Ki-67 could be used for plasma cell proliferation in stage 1 plasmacytoma due to Durie & Salmon (31), but our study found no difference of Ki-67 expression in each stage. In MM, proliferation activity was correlated to angiogenesis, and Ki-67 index was increased in the region with more vessels. This showed a correlation between Ki-67 expression and angiogenesis. The result of variant could be found due to different methods of study, patients' characteristic, population, ethnicity, and environment.(16) Girino also found no correlation in Ki-67 expression in MM cases.(32) More observation is needed to find the progression of MM cases in this study.

We found no difference between TP53 and KI-67 expression with malignancy degrees (Figure 2, Figure 3) or with age and other clinical signs. TP53 and Ki-67 expression were high in SBP and low in MM (Table 3). TP53 together with clinical stages data, Bartl histological degree was correlated with survival rate.(33) TP53 expression data can be used as a survival rate factor especially in bone marrow biopsy. Our study showed that TP53 and Ki-67 expression combined with Bartl classification were not enough to predict the development plasmacytoma into MM. Completed clinical data and stages information were needed to predict the myeloma progression. We also suggest there were other pathogenesis pathways which play roles in this progression, such as apoptosis.

SBP and EMP therapies were given for younger patients (<65 years old), similar to MM, using high doses of chemotherapy combiner with autologous stem cell transplantation (ASCT). Maintenance therapies to improve the life quality are steroid, interferon-α with or without thalidomide. Only progressed patients will continue the therapies. Asymptomatic patients won't receive any therapies but will be observed for the disease progression. (18,19) We didn't observe any therapies in this study so we couldn't know the TP53 and Bartl classification correlation to therapy responds.

Plasmacytoma survival rates depend on malignancy type and degrees. We have no sufficient data about our patients' survival rate, but we recorded only one patient died after a month ambulatory. We hope that this study can give more information that low malignancy grade (Bartl classification) MM has a better survival rate, and clinicians need to reckon Ki-67 and angiogenesis parameters for the patients.

The limitations of this study were the small number of samples and some of the samples observations only performed in small specimens of bone marrow. We also have very limited clinical data and therapies follow up due to uncompleted medical records. We suggest an integrated bone marrow observation for SBP and EMP suspected patients to confirm that has not developed into MM. Periodical check in recurrent and mortality rates also need to be observed to have a valid prognostic study.

Conclusion

Increasing expression TP53 is in accordance with Bartl's degrees of malignancy, especially in low and intermediate degree but there is no significant difference of Ki-67 proliferation indexes with Bartl's degrees of malignancy.

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