MULTIPLE MYELOMA- ANALYSIS OF DIAGNOSTIC CRITERIA AND PROGNOSTIC FACTORS

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ABSTRACT

BACKGROUND

Multiple myeloma is a plasma cell neoplasm that accounts for about 10% of haematological malignancies. It is characterised by a single clone of plasma cells producing a monoclonal protein (M protein), which results in end-organ damage resulting in hypercalcemia, renal insufficiency, anaemia, and skeletal lesions. Multiple myeloma is classified as high-risk or standard–risk disease based on fluorescence in situ hybridisation (FISH), metaphase cytogenetics and the plasma cell labelling index.

MATERIALS AND METHODS

A prospective study was conducted at Government Medical College, Thrissur, India during the period 2014-2015 with a total of 30 patients with newly diagnosed multiple myeloma. All patients were subjected to investigations like complete haemogram, ESR, serum calcium level, renal function tests, serum protein electrophoresis, β_2 microglobulin level, bone marrow trephine biopsy and skeletal survey.

RESULTS

Thirty patients enrolled in this study were analysed and compared with the existing studies. The commonest symptom was bone pain due to lytic bone lesions, seen in 73.3% of patients. Only ten patients (33%) presented with stage 1 disease, while about 60% of the patients were in the third stage of the disease, having poor prognosis, which indicates the multiple myeloma is in the late stage of the disease. β2 microglobulin was increased in 60% of patients.

CONCLUSION

In this study, about 60% of the patients are having poor prognostic features such as stage 3 disease**,** raised β_2 microglobulin, more than three lytic bone lesions. The initial staging can be quantitatively related to follow up, using tumour cell mass changes and changes in M component production. So, use of the clinical staging system provides better initial assessment and follow up of individual patients. The current approach to the diagnosis, staging, and prognosis is reviewed.

KEYWORDS

Multiple Myeloma, Electrophoresis, Staging.

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BACKGROUND

Multiple myeloma is a plasma cell neoplasm which comprises about 1% of malignant tumours and 10-15% of haemopoietic neoplasms.[1,2] Plasma cell myeloma results from expansion of a clone of immunoglobulin secreting terminally differentiated B cells. These cells will secrete a single homologous or monoclonal immunoglobulin. Plasma cell neoplasms include a spectrum of disorders ranging from monoclonal gammopathy of undetermined significance (MGUS) to plasma cell leukaemia.[3] Majority of plasma cell neoplasms are multiple myeloma, with solitary plasmacytomas accounting less than 6% of cases, and plasma cell leukaemia rarely. The incidence of multiple myeloma gradually increased during 1970- 1990, but recently there has been a plateau in the incidence from 1992 to 2008.[4] The incidence increases with increasing age, with median age of 70 years and only 2% of patients are less than 40 years of age at diagnosis.[5] There is a slight male predominance. Little is known about the cause of multiple myeloma. There are studies reporting association with prior exposure to radiation and certain chemicals such as petroleum products. It is now thought that all cases of myeloma are preceded by monoclonal gammopathy of unknown significance (MGUS), with a risk of progression to multiple myeloma of approximately 1% per year,[6,7] MGUS is present in 3-4% of general population over the age of 50 years.[8,9] Since MGUS is mostly asymptomatic and detected often as an incidental laboratory finding, only 10% of patients with newly diagnosed multiple myeloma have a history of pre-existing MGUS. Smouldering multiple myeloma is an intermediate stage between MGUS and multiple myeloma and is associated with a higher risk of progression

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of approximately 10% per year.[10] Hereditary and genetic factors may predispose to the development of myeloma. Most malignant neoplasms depend on angiogenesis for tumor progression. Recently in multiple myeloma, bone marrow angiogenesis is correlated with plasma cell labelling index and disease activity. Micro vessel density is a measure of tumor angiogenesis. Ki 67 is a proliferation marker. Micro vessel density and Ki 67 index can be used as prognostic markers in newly diagnosed cases of multiple myeloma.

Diagnosis

Patients with myeloma may present with a myriad of symptoms such as haematological manifestations, bone related problems, infections, various organ dysfunctions, neurological complaints. These signs and symptoms result from direct tumor involvement in bone marrow, effect of the protein produced by the tumor cells deposited in various organs, production of cytokines by the tumor cells or by the bone marrow microenvironment, and effects on the immune system.[11] The most common symptoms are fatigue or weakness and bone pain.[5] Fatigue is due to anaemia. Normocytic normochromic anaemia is usually observed in myeloma patients because of tumor cell involvement of bone marrow as well as inadequate erythropoietin responsiveness.[12] Other symptoms are nausea and vomiting due to renal failure and hypercalcemia, bone pain due to pathological fracture.[13] Approximately 1% to 2% of patients with multiple myeloma have extramedullary disease at the time of diagnosis and 8% have development of extramedullary disease later in the disease course.[14] Myeloma patients have high risk of developing infection and they may present with fever of unknown origin. Infection especially with gram negative bacilli is a significant cause of morbidity and death in myeloma patients.[15] Osteolytic bone lesions can be detected in approximately 80% of patients with multiple myeloma. Myeloma cells will cause coordinate increase in RANK ligand and decrease in osteoprotegerin in bone marrow which will enhance bone resorption in myeloma. Hypercalcemia is seen only in the late stage of disease. It is due to wide spread tumor induced bone destruction and impaired renal function which will cause defective clearing of excess calcium ions in the body.[16] Renal failure develops in approximately 20% cases of multiple myeloma with significant morbidity.[17] Renal impairment is caused by accumulation and precipitation of light chains which will form cast in renal tubules resulting in renal tubular obstruction. Myeloma light chain toxicity will cause damage to proximal convoluted tubule and end in renal dysfunction.[18] Malignant plasma cells will produce monoclonal antibodies which are released into the circulation.[19] This can lead to secretion of one monoclonal immunoglobulin (Ig), or free light chains or both into the serum. So, assessing M protein is important in diagnosis, disease monitoring and relapse. The traditional tests available for detection of M protein are serum and urine electrophoresis, urine Bence-Jones Protein, immunofixation electrophoresis, and serum free light chain assay.[20] Serum protein electrophoresis can be used for detection and quantification of monoclonal gammopathy. It should be recommended as screening test for all suspected cases of multiple myeloma. Immunofixation is more sensitive than serum protein electrophoresis for detecting monoclonal immunoglobulins and also to type the heavy and light chains.[21] Erythrocyte sedimentation rate is another indicator which is usually increased in multiple myeloma.[22] Bone marrow examination is an important tool in establishing diagnosis of multiple myeloma. Bone marrow aspirate and biopsy are required for proper diagnosis. Plasma cell percentage can be assessed from bone marrow aspirate. Another important advantage is that high plasma cell count can be used as a predictor of relapse in treated cases of multiple myeloma.[23] Patterns of involvement of bone marrow by plasma cells is also important. It can be diffuse, nodular, and interstitial pattern. Type of infiltration pattern is in proportion with stage of disease. Patients with plasma cells having poorly differentiated morphology had pattern of infiltration either diffuse or mixed type (nodular and interstitial). Transformation in pattern from interstitial or nodular to diffuse infiltration indicate disease progression.[24] Diagnosis of multiple myeloma was done based on International Myeloma Foundation Criteria. It has three criteria. All the three must be present for the diagnosis of multiple myeloma. The criteria are 1. Monoclonal plasma cells in the bone marrow >10% and/or a biopsy proven plasmacytoma. 2. Monoclonal protein present in the serum and/or urine. 3. Myeloma related organ dysfunction (any one or more). This includes serum calcium >10.5 mg/L or upper limit of normal, renal insufficiency, serum creatinine >2 mg/dl, anaemia- haemoglobin <10 gm/dl, lytic bone lesions or osteoporosis. The standard imaging is the skeletal survey by plain X-ray, as radionuclide bone scan usually does not detect lytic disease and has limited value.[25] It has been documented that some patients with presumed solitary plasmacytoma will be upstaged following the detection of multiple bone lesions by MRI scan or by 18-fluorine fluorodeoxyglucose positron emission tomography (FDG-PET).[26,27] The optimal role of PET in myeloma is yet to be determined but will likely evolve rapidly, and it will likely be of most benefit in non-secretory disease.[28,29]

Stage 1	Stage 2	Stage 3
All of the Following: 1. Haemoglobin >10 g/DL 2. Serum Calcium Normal 3. Normal Bone Structure or Solitary Plasmacytoma Only 4. Low M-Component (IgG <5 g/dL, IgA <3 g/dL, Urine Light Chains <4 g/24 Hours)	Fitting Neither Stage 1 or Stage 3	**One or More of the Following:** 1. Haemoglobin <8.5 g/dL 2. Serum Calcium >12 mg/dL 3. Advanced Lytic Bone Lesions 4. High M-Component (IgG >7 g/dL, IgA >3 g/dL, Urine Light Chains >12 g/24 Hours)
Table 1. Durie and Salmon Staging System		

Subclassified:

A - Relatively normal renal function (Serum Creatinine <2 mg/dL)

B - Abnormal renal function (Serum Creatinine ≥2 mg/dL)

	International Staging System	Median Survival (Months)
Stage 1	Serum β_2 microglobulin<3.5 mg/L and Serum albumin > 35 g/L	62
Stage 2	Neither 1 nor 3 i.e.; Serum β_2 microglobulin<3.5 mg/L, with S. Albumin<35 g/L, OR Serum β_2 microglobulin 3.5-5.5 mg/L	44
Stage 3	Serum β_2 microglobulin>5.5 mg/L	29
Table 2. International Staging System		

Prognosis and Risk Stratification

Patients with multiple myeloma have variable disease courses, with survival ranging from less than 1 year to more than 10 years, depending on host factors, tumor burden (stage), biology and response to therapy.[30] Evaluation of prognostic factors is important to define therapeutic strategies, and predict life expectancy after diagnosis. Tumor burden in multiple myeloma has traditionally been assessed using the Durie- Salmon staging system,[31] which was predictive of clinical outcomes after standard –dose chemotherapy. Tumor burden related prognostic factors are high β_2 microglobulin (β_2 M>2.5 mg/L), >3 lytic bone lesions, haemoglobin<8.5 gm/dl, serum calcium >12 mg/dl. Among this β2-microglobulin >2.5 mg /L has been identified as one of the most consistent predictors of survival in plasma cell myeloma. High β2 microglobulin levels carry poor prognosis for treatment with both standard dose and high dose chemotherapy.[32] Tumor microenvironment-related prognostic factors are bone marrow microvessel density, soluble CD16, and serum syndecan-1 levels. Cytogenetic abnormalities have been identified as a major prognostic factor in multiple myeloma. Detection of any cytogenetic abnormality such as deletion of chromosome 13, presence of the t (4;14) translocation is considered to suggest higher risk disease.[33] Other independent factors associated with poor prognosis include elevated serum lactate dehydrogenase (LDH), elevated C-reactive protein, soluble IL-16 receptor, IgA myeloma. Other patient related prognostic factors are age, albumin and performance status. Serum albumin >3.5gm/dl is associated with good prognosis. Age >70 years and performance status 3 or 4 carry poor prognosis. International Staging System (ISS) has been validated to assist in prognostication based on two variables such as serum albumin and β2 microglobulin.[34]

Aim of Study

The present study is aimed to analyse newly diagnosed multiple myeloma cases in terms of symptoms, diagnostic criteria, staging, and prognostic factors and correlating with each other.

MATERIALS AND METHODS

This prospective study was conducted at Government Medical College, Thrissur, Kerala during the period of February 2014 to August 2015. The aim of the study is to analyse the symptoms, diagnostic criteria, staging and prognostic factors and correlating with each other. 30 newly diagnosed cases of multiple myeloma are included in this study. The data were collected using a proforma. This includes age, sex, symptoms, haemoglobin, ESR, peripheral smear, bone marrow aspiration and trephine biopsy. Biochemical investigations include renal function test, serum calcium level, serum lactate dehydrogenase (LDH), serum C-reactive protein, total protein, albumin, serum protein electrophoresis, urine Bence-Jones protein. Radiological investigations included plain X-ray, CT scan and MRI scan. Bone marrow aspiration and trephine biopsy were done under strict aseptic precautions. Bone marrow aspirate and imprint smears were fixed in methanol. Later staining done with Romanowsky stain. Both Leishman and Wright's staining were done. Bone marrow trephine biopsy specimens were fixed in Bouin's fixative and decalcified in 5% nitric acid. Biopsy was processed, and sections were taken from paraffin embedded tissues. Sections were stained with haematoxylin and eosin and studied under light microscopy. In the trephine biopsy, the distribution of plasma cells was noted as diffuse, interstitial, nodular, focal, paratrabecular and combined. CD34 immunohistochemistry was done on bone marrow biopsy sections to stain endothelial cells. Microvessel density was calculated using image analysis

software VM3.6. Staging of the disease was done based upon Durie and Salmon criteria. Data was collected, and statistical analysis was done using software SPSS version 16.

RESULTS

A total of 30 cases of multiple myeloma were studied. Out of 30 cases 18(60%) were females. Mean age was 59.1 years. Most frequent age group was 41-60 years (73.3%). There were no patients below 40 years. Performance status of 15 patients (50%) were 3, 8 patients (26.4%) 4 and 7 patients (23.3%) 2. Out of multiple symptoms bone pain was the commonest symptom seen in 90% (27 patients), followed by pallor in 50% (15 patients), and fever 26.6% (8 cases). 33.3% (10 cases) patients had haemoglobin >10 gm/dl and 76.6% (20 cases) had haemoglobin <10 gm/dl. Hypercalcemia is seen in 6 cases (20%). Out of 30 cases, 7 cases (23.3%) had renal impairment with serum creatinine >2 mg/dl and 23 cases (76.7%) had normal serum creatinine level. Out of 30 cases studied 19 cases (63.3%) had normocytic normochromic anaemia. 3 cases (10%) had associated thrombocytopenia. 3 cases (10%) had pancytopenia. 3 cases (10%) had leukoerythroblastic blood picture. 5 cases (16.7%) had normocytic normochromic blood picture. Pattern of distribution of plasma cells in bone marrow trephine biopsy are given in the table no. 3.

Pattern of Involvement	Frequency	Percentage
Diffuse	13	43.3%
Interstitial	6	20%
Diffuse and Interstitial	8	26.7%
Focal/Nodular	2	6.7%
Paratrabecular	1	3.3%
Total	30	100%

Table 3

In this study 29 cases (96.7%) had monoclonal protein (M band) on serum protein electrophoresis. One case did not have M band on serum protein electrophoresis. Bone lytic lesions were present in 22 patients (73.3%). Out of 30 cases 60% (18 cases) had increased serum β2 microglobulin level (>3.5 mg/dl). ESR was <50 mm/hr in 7 cases (23.3%), between 50 and 100 in 8 cases (26.6%), and >100 in 15 cases (50%). Lactate dehydrogenase is elevated in 16 patients (53.3%), and C-reactive protein is elevated in 21 patients (69.9%). According to Durie and Salmon staging 10 cases (33.3%) were in stage 1, 2 cases (6.6%) were in stage 2 and 18 cases (60%) were in stage 3. Each stage is divided into A and B depending upon the level of serum creatinine as given in the figure no 1. Microvessel density is assessed using CD34 staining of endothelial cells, which is given in the figure No. 2. Microvessel density range from 1-13.6/0.04 mm2 area (Mean-5.57/0.04 mm2 & standard deviation - 3.281).

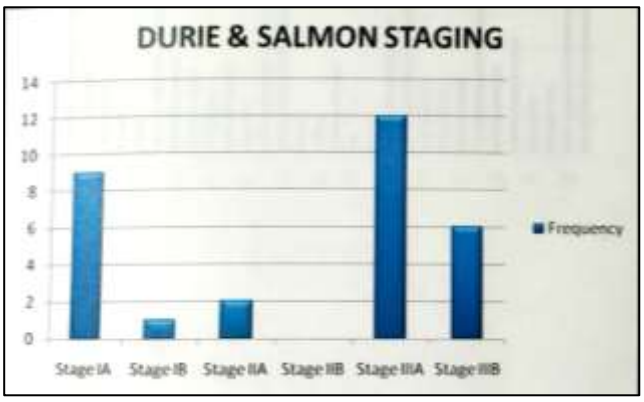

Figure 1

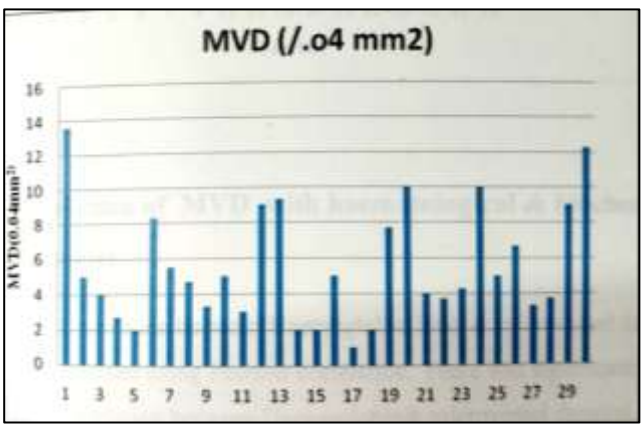

Figure 2

DISCUSSION

Major advances in the diagnosis and treatment of multiple myeloma have occurred in the last decade. The diagnosis requires the presence of monoclonal bone marrow plasma cells >10%, serum and/or urinary M protein and evidence of end organ damage (CRAB- hypercalcemia, renal disease, anaemia, or lytic bone lesions). In this study age of the patients range from 42-89 years.[24] Clinical presentation of 27 cases in this study were suspicious of multiple myeloma. These patients had bone pain and on radiological examination bone lytic lesions were present in 22 patients at the time of diagnosis.[13] Skull and spine were the most commonly affected sites. Other studies also reported bone destruction in 76- 80% of cases.[35,36] In this study 20 patients (66.6%) had anaemia and 3 patients (10%) had pancytopenia at the time of diagnosis.[37] Out of 30 cases only 6 cases (20%) had hypercalcemia.

In this study 96.7% (29 patients) had M band in serum protein electrophoresis. But in some other studies only 9-10% cases had M band in serum protein electrophoresis.[21,38] Out of 30 cases 22 cases had IgG as the monoclonal immunoglobulin, 6 cases had IgM, and 2 cases had kappa light chain as monoclonal protein. β_2 microglobulin level was increased in 18 cases (60%) which is the single most important prognostic factor. About 60% of the patients are having elevated LDH, and C-reactive protein indicates poor prognosis. Out of these 7 cases (23%) had renal impairment also, and these cases belong to stage 3 disease. So high serum β2 microglobulin level and renal impairment correlated with poor prognosis.[39,40] In this study microvessel

density was assessed using CD34 immunostaining for endothelial cells. There was a significant positive correlation with stage of the disease and microvessel density (P value<0.05), and Ki 67 index. Microvessel density was more in higher stage disease (Stage 3).[41,42]

CONCLUSION

In this study, current approach to diagnosis, staging, and prognosis were reviewed. Staging was done according to Durie and Salmon criteria and about 2/3rd of the patients were in the third stage of disease at the time of diagnosis. This indicates multiple myeloma was in the late stage of disease and indiacted poor prognosis. Although the Durie and Salmon staging system has several shortcomings, we believe that it remains useful in comparing patients in clinical trials and allows a better assessment of the disease burden of patients in a given study. It is now possible to classify multiple myeloma as standard-risk and high-risk depending on certain independent factors such as metaphase cytogenetics, fluorescence in situ hybridisation (FISH), and the plasma cell labelling index, which are not included in this study due to constraints in logistics.

REFERENCES

[1] Rajkumar SV. Treatment of multiple myeloma. Nat Rev Clin Oncol 2011;8(8):479-491.

[2] Rajkumar SV, Gahrton G, Bergsagel PL. Approach to the treatment of multiple myeloma: a clash of philosophies. Blood 2011;118(12):3205-3211.

[3] Swerdlow SH, Campo E, Harris NL, et al. Plasma cell neoplasms. In: WHO classification of tumours of haematopoietic and lymphoid tissues. 4th edn. IARC Lyon 2008:200-209.

[4] Kyle RA, Therneau TM, Rajkumar SV, et al. Incidence of multiple myeloma in Olmsted County, Minnesota: Trend over 6 decades. Cancer 2004;101(11):2667-2674.

[5] Kyle RA, Gertz MA, Witzig TE, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clinic Proc 2003;78:21-33.

[6] Kyle RA, Therneau TM, Rajkumar SV, et al. A long term study of prognosis of monoclonal gammopathy of undetermined significance. N Engl J Med 2002;346(8):564-569.

[7] Landgren O, Kyle RA, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. Blood 2009;113(22):5415-5417.

[8] Kyle RA, Therneau TM, Rajkumar SV, et al. Prevalence of monoclonal gammopathy of undetermined significance. N Engl J Med 2006;354(13):1362-1369.

[9] Dispenzieri A, Katzmann JA, Kyle RA, et al. Prevalence and risk of progression of light-chain monoclonal gammopathy of undetermined significance: a retrospective population based cohort study. Lancet 2010;375(9727):1721-1728.

[10] Kyle RA, Remstein ED, Therneau TM, et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. N Engl J Med 2007;356(25):2582-2590.

[11] van Rhee F, Anaissie E, Angtuaco E, et al. Myeloma. Chap- 109. In: Kenneth K, Ernest B, Uri S, et al, eds. Williams hematology. 8th edn. McGraw-Hill Education 2010:1198-1225.

[12] Rokicka PM, Paszkowska M, Krol M, et al. Analysis of causes for anemia in patients with multiple myeloma. Pol Arch Med Wewn 2000;104(6):843-851.

[13] Singhal S, Mehta J. Multiple myeloma. Clin J Am Soc Nephrol 2006;1(6):1322-1330.

[14] Short KD, Rajkumar SV, Larson D, et al. Incidence of extramedullary diseasein patients with multiple myeloma in the era of novel therapy, and the activity of pomalidomide on extramedullary myeloma. Leukemia 2011;25(6):906-908.

[15] Savage DG, Lindenbaum J, Garrett TJ. Biphasic pattern of bacterial infection in multiple myeloma. Ann Intern Med 1982;96(1):47-50.

[16] Kyle RA. Multiple myeloma: review of 869 cases. Mayo Clin Proc 1975;50(1):29-40.

[17] Goldschmidt H, Lannert H, Bommer J, et al. Multiple myeloma and renal failure. Nephrol Dial Transplant 2000;15(3):301-304.

[18] Dimopoulos MA, Kastritis E, Rosinol L, et al. Pathogenesis and treatment of renal failure in multiple myeloma. Leukemia 2008;22(8):1485-1493.

[19] Mehta KD, Khambu B, Lakhey M, et al. Diagnosis of multiple myeloma by demonstration of M protein in bone marrow aspirate by agar gel electrophoresis: a case report. Kathmandu Univ Med J 2006;4(4):513-516.

[20] Ludwig H, Miguel JS, Dimopoulos MA, et al. International myeloma working group recommendations for global myeloma care. Leukemia 2014;28(5):981-992.

[21] Tripathy S. The role of serum protein electrophoresis in the detection of multiple myeloma: an experience of a corporate hospital. J Clin Diagn Res 2012;6(9):1458-1461.

[22] San Miguel JF, Gutierrez NC, Mateo G, et al. Conventional diagnostics in multiple myeloma. Eur J Cancer 2006;42(11):1510-1519.

[23] Rajkumar SV, Fonseca R, Dispenzieri A, et al. Methods for estimation of bone marrow plasma cell involvement in myeloma: predictive value for response and survival in patients undergoing autologous stem cell transplantation. Am J Hematol 2001;68(4):269-275.

[24] Stifter S, Babarovic E, Valkovic T, et al. Combined evaluation of bone marrow aspirate and biopsy is superior in the prognosis of multiple myeloma. Diagn Pathol 2010;5:30.

[25] Durie BG, Kyle RA, Belch A, et al. Myeloma management guidelines: a consensus report from the scientific advisors of the international myeloma foundation. Hematol J 2003;4(6):379-398.

[26] Mariette X, Zagdanski AM, Guermazi A, et al. Prognostic value of vertebral lesions detected by

magnetic resonance imaging in patients with stage I multiple myeloma. Br J Haematol 1999;104(4):723-729.

[27] Van de Berg BC, Lecouvet FE, Michaux L, et al. Stage I multiple myeloma: value of MR imaging of the bone marrow in the determination of prognosis. Radiology 1996;201(1):243-246.

[28] Schirrmeister H, Buck AK, Bergmann L, et al. Positron emission tomography (PET) for staging of solitary plasmacytoma. Cancer Biother Radiopharm 2003;18(5):841-845.

[29] Kim PJ, Hicks RJ, Wirth A, et al. Impact of 18F-fluorodeoxyglucose positron emission tomography before and after definitive radiation therapy in patients with apparently solitary plasmacytoma. Int J Radiat Oncol Biol Phys 2009;74(3):740-746.

[30] Russell SJ, Rajkumar SV. Multiple myeloma and the road to personalised medicine. Lancet Oncol 2011;12(7):617-619.

[31] Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 1975;36(3):842-854.

[32] Attal M, Harousseau JL, Facon T, et al. Single versus double autologous stem-cell transplantation for multiple myeloma. N Engl J Med 2003;349(26):2495-2502.

[33] Chang H, Sloan S, Li D, et al. The t (4; 14) is associated with poor prognosis in myeloma patients undergoing autologous stem cell transplant. Br J Haematol 2004;125(1):64-68.

[34] Greipp PR, San Miguel J, Durie BG, et al. International staging system for multiple myeloma. J Clin Oncol 2005;23(15):3412-3420.

[35] Sutandyo N, Firna E, Agustina J, et al. Clinicopathology profile and bone involvement of multiple myeloma in Dharmais National Cancer Hospital, Indonesia. Asian Pac J Cancer Prev 2015;16(15):6261-6265.

[36] Blade J, Kyle R, Greipp PR. Presenting features and prognosis in 72 patients with multiple myeloma who were younger than 40 years. Br J Haematol 1996;93(2):345-351.

[37] Khodke K, Marwah S, Buxi G, et al. Bone marrow examination in cases of pancytopenia. JIACM 2001;2(1&2):55-59.

[38] Chopra GS, Guptha PK, Mishra DK. Evaluation of suspected monoclonal gammopathies: experience in a tertiary care hospital. Med J Armed Forces India 2006;62(2):134-137.

[39] Qin X, Xu Y, An G, et al. The impact of renal function on prognostic value of β_2-microglobulin of ISS stage system in multiple myeloma patients--Clinical data analysis of 666 patients in a single center. Zhonghua Xue Ye Xue Za Zhi 2015;36(5):393-397.

[40] D'Anastasi M, Notohamiprodjo M, Schmidt G, et al. Tumor load in patients with multiple myeloma: β2-microglobulin levels versus whole body MRI. Am J Roentgenol 2014;203(4):854-862.

[41] Alexandrakis MG, Passam FH, Dambaki C, et al. The relation between bone marrow angiogenesis and the proliferation index Ki-67 in multiple myeloma. J Clin Pathol 2004;57(8):856-860.

[42] Ahn MJ, Park CK, Choi JH, et al. Clinical significance of micro-vessel density in multiple myeloma patients. J Korean Med Sci 2001;16(1):45-50.