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Clinical and laboratory significance of CD56 (a neural cell adhesion molecule) positivity in multiple myeloma and AL amyloidosis

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CD56 is frequently expressed by malignant plasma cells in patients with multiple myeloma (MM) or AL amyloidosis (ALA). The aim of this retrospective study was to examine CD56 expression in patients with MM or ALA and determine the clinical and laboratory characteristics associated with its expression. Bone marrow samples of patients were analyzed by flow cytometry. Various clinical data with regard to the expression or non-expression of CD56 were compared. In total, 316 patients (215 with MM and 101 with ALA) were included in the study. The CD56 expression rate was higher in patients with MM than that in those with ALA. Patients with MM had a median CD56 expression rate of 96.4%, and serum total protein levels were significantly higher in the CD56+ group ($p < 0.001$). Patients with ALA had a median CD56 expression rate of 1.9%, and serum lactate dehydrogenase levels were significantly higher in the CD56+ group ($p = 0.033$). These findings suggest that the CD56 expression rate differs in monoclonal plasma cells between patients with MM and ALA, and that M-protein secretions are increased in patients with CD56+ MM.

Key words: multiple myeloma, AL amyloidosis, CD56, NCAM

Introduction

CD56 (a neural cell adhesion molecule, NCAM) is a membrane glycoprotein belonging to the immunoglobulin superfamily [1]. Normal plasma cells do not express CD56, but it is frequently expressed by malignant plasma cells in patients with multiple myeloma (MM) or AL amyloidosis (ALA) [2,3]. CD56 is expressed in approximately 65% of MM cases and may be associated with osteolysis, good prognosis. CD56 negativity may be associated with poor prognosis, extramedullary involvement, plasmablastic morphology, plasma cell leukemic state, and non-hyperdiploidy chromosomal abnormality. However, these findings have been questioned, and a previous prospective study reported that patients with CD56– MM presented a lower frequency of osteolysis, while they were not associated with a poor prognosis [4].

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CD56 is expressed in approximately 50% of ALA cases [3]. In patients with ALA, the number of neoplastic plasma cells is usually lesser and light chain restriction is less reliable because the normal plasma cells are not suppressed. CD56 expression is helpful in identifying neoplastic plasma cells in ALA. Unlike MM, the clinicopathological correlation between CD56 expression and ALA remains poorly understood. By flow cytometry, a small number of neoplastic plasma cells from patients with ALA can be analyzed rapidly [5].

The difference in CD56 expression rates between MM and ALA suggests differences in the pathogenesis of these diseases, but to the best of our knowledge, such a study has not yet been reported. The aim of this retrospective study was to examine CD56 expression in patients with MM and ALA by flow cytometry in order to define the clinical characteristics associated with CD56 expression.

Materials and Methods

Patients

This retrospective study included 215 patients with MM and 101 patients with ALA who underwent bone marrow examination at the Department of Hematology of the Japanese Red Cross Medical Center in Tokyo, Japan, from May 2006 to December 2012.

The diagnosis of MM or ALA was obtained from the hospital medical records. Various clinical and laboratory data, including age; sex; bone marrow examination; monoclonal typing; serum concentrations of immunoglobulins, total protein, albumin, calcium, lactate dehydrogenase, uric acid, and beta-2 microglobulin at the bone marrow examination; and the clinical course were also obtained from the hospital medical records. These data were compared with regard to CD56 expression. Approval was obtained from the Institutional Review Board of the Japanese Red Cross Medical Center, Tokyo, Japan, exempting the study from requiring consent from individual patient's consent.

Flow cytometry

Bone marrow aspirate samples were analyzed using a flow cytometer (FACSCalibur, Becton Dickinson and Company, Tokyo, Japan). The following antibodies were used for flow cytometry: CD38 (Becton Dickinson and Company, Tokyo, Japan), CD138 (Beckman Coulter, Tokyo, Japan), CD56 (Becton Dickinson and Company, Tokyo, Japan), and CD19 (Beckman Coulter, Tokyo, Japan). Twenty thousand cells were analyzed to determine the percentage of bone marrow cells.

Plasma cells were identified by gating using CD38. Next the gate was set for plasma cells and the expression levels of CD56, CD19, and CD138 were analyzed. The percentage of plasma cells expressing CD56 was evaluated by flow cytometry. Patients were considered to be positive for CD56 when its expression was detected in more than 50% of tested plasma cells.

Statistical analysis

Clinical data were evaluated with regard to CD56 expression using the Shapiro–Wilk test, Levene's test, Student's *t*-test, or Mann–Whitney test. Survival analysis was performed by the Kaplan–Meier method, and comparisons of survival rates were evaluated by the log-rank test. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS v.18.0 (IBM Corp., Armonk, NY, USA).

Results

The characteristics of the 316 patients are shown in Tables 1 and 2. The median ages of patients with MM and ALA were 65 years and 64 years, respectively. The information of the type of M-protein type was available in 88% in patients with MM and 72% of the patients with ALA. By flow cytometry, the median ratio of CD38+ plasma cells in bone marrow nucleated cells were 13.6% (range, 0.1%–87.0%) for MM and 1.9% (range, 0.1%–23.1%) for ALA. Typical phenotypes of CD38+ plasma cells in patients with MM and ALA are shown in Figures 1 and 2, respectively.

Table 1. Characteristics of patients with multiple myeloma

Multiple myeloma (n = 215)	
Gender	
Male	95
Female	120
Age (years)	
Median	65
Range	29–88
Monoclonal protein	
IgG kappa	75
IgG lambda	44
IgA kappa	25
IgA lambda	11
IgD kappa	2
IgD lambda	2
BJ kappa	19
BJ lambda	12
Non-secretory	1
Not available	24
Percentage of CD38+ plasma cells (%)	
Median	13.6
Range	0.1–87.0

Table 2. Characteristics of patients with AL amyloidosis

AL amyloidosis (n = 101)	
Gender	
Male	56
Female	45
Age (years)	
Median	64
Range	39–84
Monoclonal protein	
IgG kappa	3
IgG lambda	24
IgA kappa	2
IgA lambda	9
IgM kappa	2
IgM lambda	2
Biclonal	1
IgG kappa and IgA kappa	1
BJ kappa	8
BJ lambda	22
Not determined	28
Percentage of CD38+ plasma cells (%)	
Median	1.9
Range	0.1–23.1

CD56+ plasma cells were observed in 72.6% (155/215) of patients with MM and in 42.6% (43/101) of patients with ALA (Figs. 1 and 2). Among patients with MM (n = 215), the median ratio of CD56+ plasma cells was 96.4% (range, 0%–100%).

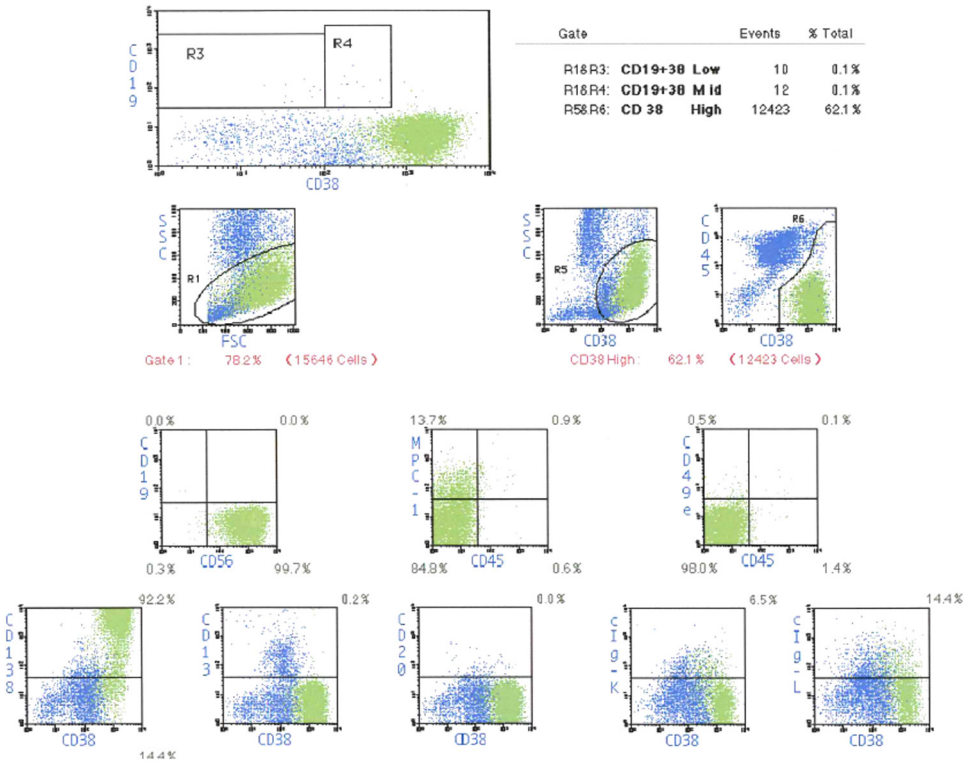

Figure 1. Typical phenotype of CD38+ plasma cells of a patient with multiple myeloma. A few CD19+ normal plasma cells were present. Most cells were CD56+ abnormal plasma cells.

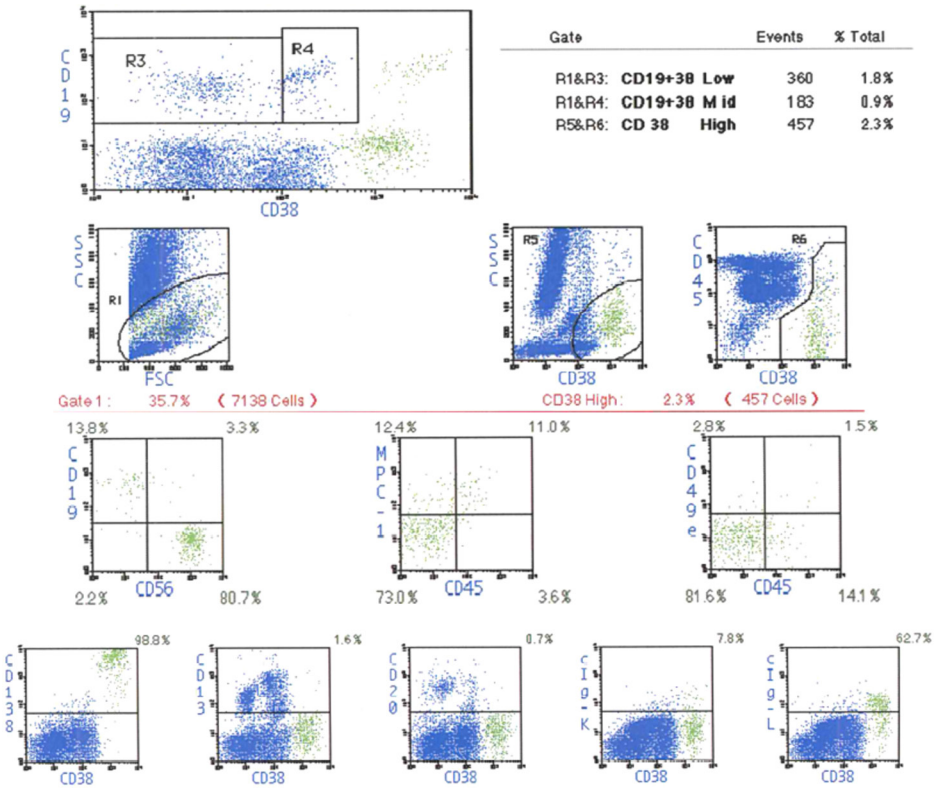

Figure 2. The typical phenotype of CD38+ plasma cells of a patient with AL amyloidosis. A few CD19+ normal plasma cells were present. Most cells were CD56+ abnormal plasma cells. CD20 expression also was observed.

Table 3. Comparison of disease characteristics between patients with CD56+ and CD56– multiple myeloma

	CD56+ cases (n = 155)	CD56– cases (n = 60)	p-value
Gender			
Male	68	27	0.081
Female	87	33	
Age (years)			
Median	65.0	64.5	0.477
Range	31–88	29–88	
Serum total protein (g/dL)			
Median	8.4	6.7	<0.001
Range	4.0–15.1	4.7–13.5	
Serum albumin (g/dL)	3.7 2.0–5.0	3.9 2.2–4.8	0.052
Serum calcium (mg/dL)	9.0 7.2–14.3	8.47 7.2–11.9	0.077
Serum lactate dehydrogenase (IU/L)	187.0 85–3337	199.5 104–4922	0.061
Serum beta 2-microglobulin (mg/L)	3.35 1.0–61.6	2.65 1.1–42.3	0.061
Bone fracture at diagnosis	29	15	0.306

A comparison of the disease characteristics for patients with CD56+ and CD56– MM is presented in Table 3. Data for serum calcium, uric acid, and beta 2-microglobulin were unavailable for all patients. The serum total protein levels were significantly higher in the CD56+ group than those in the CD56– group ($p < 0.001$). Although serum albumin and lactate dehydrogenase levels were lower and serum calcium and beta 2-microglobulin levels were higher in the CD56+ group than those in the CD56– group, these differences were insignificant. In addition, there were no differences in the uric acid levels between the two groups. Bone fractures at the diagnosis of MM were observed in 18.7% (29/155) of the CD56+ patients and 25% (15/60) of the CD56– patients. However, this difference was insignificant ($p = 0.306$). The median survival of the 215 patients after diagnosis was 79 months, and those of the CD56+ MM group and CD56– MM group were 79 months and 86 months, respectively. The survival of patients in both groups showed no significant differences ($p = 0.809$; Fig. 3).

Among patients with ALA (n = 101), the median ratio of CD56+ cells was 33% (range, 1.2%–99.9%). The CD56–/CD19+ expression rate was 8.4% (range 0%–93.3%).

A comparison of the disease characteristics of patients with CD56+ and CD56– ALA is presented in Table 4. The data for beta 2-microglobulin were not available in some patients with ALA. Serum lactate dehydrogenase levels were significantly higher in the CD56+ group than those in the CD56– group ($p = 0.033$), whereas no differences were observed in total protein, albumin, calcium, uric acid, and beta-2 microglobulin

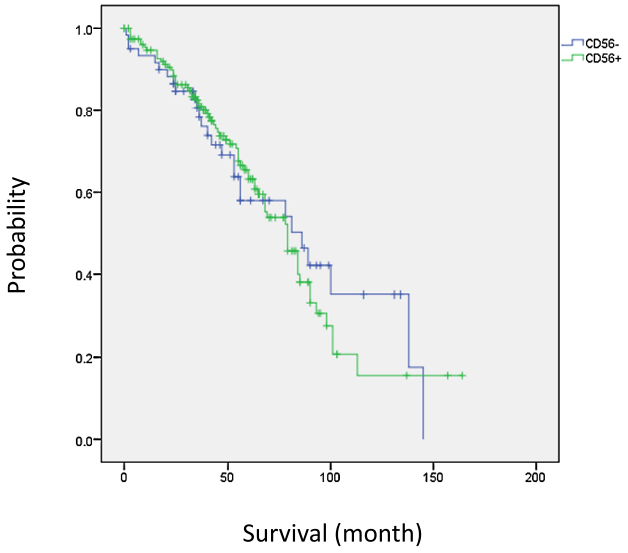

Figure 3. Overall survival after the diagnosis of CD56 positive and negative patients with multiple myeloma. Median survival in these patients was 79 months and 86 months, respectively ($p = 0.809$).

levels between these two groups. The percentages of patients diagnosed with heart failure, nephrotic syndrome, carpal tunnel syndrome, and hepatomegaly were 37.2% (16/43), 39.5% (17/43), 9.3% (4/43), and 4.7% (2/43), respectively, in the CD56+ ALA group, and 34.5% (20/58), 41.4% (24/58), 1.7% (1/58), and 0%, respectively, in the CD56– ALA group, with no significant differences between the groups ($p = 0.778$, 0.853, 0.084, and 0.099, respectively). The median survival among

Table 4. Comparison of disease characteristics between patients with in CD56+ and CD56– AL amyloidosis

	CD56+ cases (n = 43)	CD56– cases (n = 58)	p-value
Gender			
Male	19	37	0.051
Female	24	21	
Age (years)			
Median	63.0	64.5	0.247
Range	39–76	39–84	
Serum total protein (g/dL)			
Median	5.4	5.8	0.116
Range	3.5–7.1	2.9–7.7	
Serum albumin (g/dL)	3.5 1.9–4.4	3.5 1.7–4.9	0.240
Serum calcium (mg/dL)	8.6 7.1–9.9	8.47 7.0–10.7	0.077
Serum lactate dehydrogenase (IU/L)	255.0 109–487	218.0 88–540	0.033
Serum beta 2-microglobulin (mg/L)	2.4 0.9–6.8	2.5 0.8–13.9	0.912
Heart failure at diagnosis	16	20	0.778
Nephrotic syndrome at diagnosis	17	24	0.853
Carpal tunnel syndrome at diagnosis	4	1	0.084
Hepatomegaly at diagnosis	2	0	0.099

patients in the CD56+ ALA group and CD56– ALA group were 70 months and 118 months, respectively. However, this difference was not statistically significant (p = 0.582; Fig. 4).

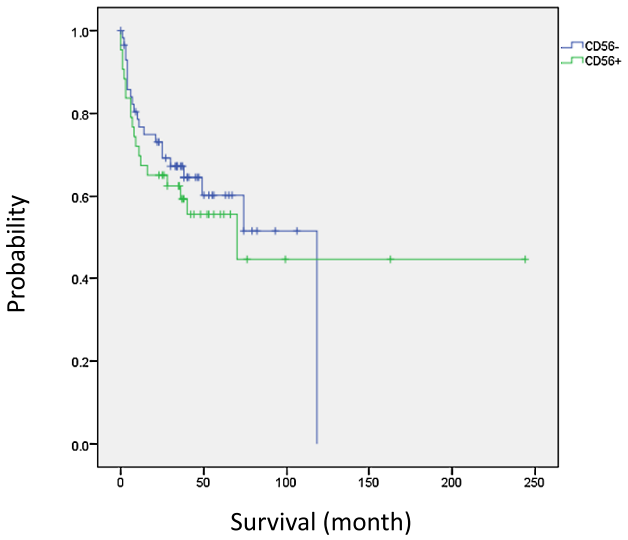

Figure 4. Overall survival after the diagnosis of CD56 positive and negative patients with AL amyloidosis. Median survival in these patients was 70 months and 118 months, respectively (p = 0.582).

Discussion

CD56+ plasma cells were observed in 72.6% of patients with MM and in 42.6% of patients with ALA. These findings are consistent with those reported previously [4–8]. Abnormal CD56 expression is well described in MM, with many reports demonstrating a correlation with CD56 expression. Because CD56 is an adhesion molecule, CD56 positivity may contribute to plasma cell-to-bone trabecular interactions [9]. However, data regarding plasma cell characteristics in ALA are limited [7]. A previous series of 36 cases reported a CD56 expression rate of 50% based on immunohistochemical analysis [3]. To the best of our knowledge, the present study reports the largest number of ALA cases (101 cases) to date. Because abnormal plasma cells were examined by the same method for both ALA and MM, the CD56 expression rate was considered lower for ALA than that for MM. The low CD56 expression rate in ALA plasma cells suggests weak plasma cell-to-bone trabecular interactions.

In this study, patients with CD56+ MM had higher serum total protein levels than patients with CD56– MM. A highly significant correlation between CD56 expression and paraprotein levels in patients with MM was reported previously [10]. In the same study, CD56 expression, however, was reportedly inversely correlated with serum albumin levels. Although, in

the present study, serum albumin levels were lower in patients with CD56+ MM, the difference was not statistically significant. M protein secretions may be increased in patients with CD56+ MM, and the CD56 negativity of ALA plasma cells may be related to a lower M protein (particularly, heavy chain) secretion, but these assumptions require further investigation. In a previous report, patients with CD56– MM were found to have higher beta-2 microglobulin levels [11], but this was not consistent with our findings.

Variable results regarding bone lesions have been reported [4,11]. In our study, bone fractures rather than lytic bone lesions were evaluated, but no significant difference was observed. This suggests CD56 expression is not related to bone lesions or bone fractures. Previous studies have reported that CD56 negativity in MM was associated with poorer outcome [4,11], but other studies and our study did not suggest these differences. CD56 expression may not be a major determinant of prognosis.

Information regarding the clinicopathological significance of CD56 in ALA is very limited. In this study, lactate dehydrogenase levels were significantly higher in patients with CD56+ ALA than those in patients with CD56– ALA. This paradox was observed in MM cases, in which serum lactate dehydrogenase levels were lower in the CD56+ cases than those in the CD56– cases ($p = 0.061$). In previous studies, serum lactate dehydrogenase was not significantly different in MM [4,11]. This suggests that CD56+ ALA is associated with the destruction of many cells, because lactate dehydrogenase is found in various tissues including heart, blood cells, skeletal muscles, liver, and is elevated when these cells are broken. However, the clinical significance of this difference observed in ALA is questionable and should be clarified in further studies.

Heart failure at diagnosis of ALA was observed in 37.2% and 34.5% of CD56+ and CD56– patients, respectively, in our study, with no significant difference between the groups. Heart failure suggesting the cardiac deposition of amyloid in ALA patients is strongly associated with poor prognosis. The survival of ALA patients in this study was much better than expected [12]. This might be due to the relatively low rate of cardiac involvement. Cardiac involvement was observed in approximately 60% of patients in a previous study [13]. However, the survival rate was not significantly different according to CD56 expression. In addition, the incidence rates of nephrotic syndrome, carpal tunnel syndrome, and hepatomegaly were not significantly different with regard to CD56 expression. These findings suggest that in ALA, CD56 expression is not related to the "dominant" site of deposition. The limitations of this study were its retrospective design and the inclusion of both treated and untreated patients.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

References

1) Cunningham BA, Hemperly JJ, Murray BA, Prediger EA, Brackenbury R, Edelman GM. Neural cell adhesion molecule: structure, immunoglobulin-like domains, cell surface modulation, and alternative RNA splicing. Science. 1987; 236: 799–806.
2) Van Camp B, Durie BG, Spier C, De Waele M, Van Riet I, Vela E, et al. Plasma cells in multiple myeloma express a natural killer cell-associated antigen: CD56 (NKH-1; Leu-19). Blood. 1990; 76: 377–82.
3) Deshmukh M, Elderfield K, Rahemtulla A, Naresh KN. Immunophenotype of neoplastic plasma cells in AL amyloidosis. J Clin Pathol. 2009; 62: 724–30.
4) Kraj M, Sokołowska U, Kopeć-Szlezak J, Pogłód R, Kruk B, Woźniak J, et al. Clinicopathological correlates of plasma cell CD56 (NCAM) expression in multiple myeloma. Leuk Lymphoma. 2008; 49: 298–305.
5) Matsuda M, Gono T, Shimojima Y, Hoshii Y, Ikeda S. Phenotypic analysis of plasma cells in bone marrow using flow cytometry in AL amyloidosis. Amyloid. 2003; 10: 110–6.
6) Robillard N, Wuillème S, Moreau P, Béné MC. Immunophenotype of normal and myelomatous plasma-cell subsets. Front Immunol. 2014; 5: 137.
7) Kumar S, Kimlinger T, Morice W. Immunophenotyping in multiple myeloma and related plasma cell disorders. Best Pract Res Clin Haematol. 2010; 3: 433–51.
8) Chang H, Samiee S, Yi QL. Prognostic relevance of CD56 expression in multiple myeloma a study including 107 cases treated with high-dose melphalan-based chemotherapy and autologous stem cell transplant. Leuk Lymphoma. 2006; 47: 43–7.
9) Kaiser U, Auerbach B, Oldenburg M. The neural cell adhesion molecule NCAM in multiple myeloma. Leuk Lymphoma. 1996; 20: 389–95.
10) Kaiser U, Oldenburg M, Jaques G, Auerbach B, Havemann K. Soluble CD56 (NCAM): a new differential-diagnostic and prognostic marker in multiple myeloma. Ann Hematol. 1996; 73: 121–6.
11) Sahara N, Takeshita A, Shigeno K, Fujisawa S, Takeshita K, Naito K, et al. Clinicopathological and prognostic characteristics of CD56-negative multiple myeloma. Br J Haematol. 2002; 117: 882–5.
12) Kumar S, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, Colby C, et al. Revised prognostic staging system for light chain amyloidosis incorporating cardiac biomarkers and serum free light chain measurements. J Clin Oncol. 2012; 30: 989–95.
13) Kyle RA, Gertz MA. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. Semin Hematol. 1995; 32: 45–59.