Antigen Expression Patterns of Plasma Cell Myeloma: An Association of Cytogenetic Abnormality and International Staging System (ISS) for Myeloma

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Background: Immunophenotyping of plasma cell has become an important diagnostic tool for plasma cell myeloma. There have been a few studies for association of antigen expression and cytogenetic abnormality of plasma cell myeloma. **Methods:** A total of 68 symptomatic/smoldering plasma cell myeloma case were analyzed by multicolor flow cytometry using CD38 and CD138 for primary gating of plasma cells. A conventional cytogenetics and fluorescence in situ hybridization (FISH) studies for detection of del(13q) or aneuploidy, del(17p), and IGH/FGFR translocation were done. We statistically analyzed the association of antigen expression and cytogenetic abnormality/myeloma stage (international stag-

ing system for multiple myeloma). **Results:** Positive expression of CD19, CD28, CD45, CD56, CD117, and CD274 was detected in 8.8%, 50.0%, 50.0%, 75.0%, 39.7%, and 2.9% of cases, respectively. CD117-negative cases were associated with hypodiploidy ($P = 0.017$). CD45-negative cases were associated with deletion 13 or aneuploidy ($P < 0.001$) and del(17p) ($P = 0.011$) by FISH. CD45-negativity or CD117-negativity was associated with advanced stage ($P = 0.012$ and $P = 0.016$, respectively). **Conclusion:** The antigen expression patterns of myeloma plasma cell were associated with cytogenetic abnormality and stage. J. Clin. Lab. Anal. 29:505–510, 2015. © 2014 Wiley Periodicals, Inc.

Key words: myeloma; flow-cytometry; cytogenetic abnormality

INTRODUCTION

The antigenic expression patterns of plasma cell myeloma and normal plasma cell are different. Lack of CD19 expression and aberrant expression of CD28, CD117, CD56, and CD33 are common findings of plasma cell myeloma (1, 2). Therefore flow cytometry are used for diagnosis of plasma cell myeloma (3). In addition to diagnostic purpose, several studies have shown prognostic value of antigen expression patterns (4–7). However, cytogenetic abnormalities and international staging system (ISS) is most important prognostic indicator in myeloma (8–10). The del(13q) or aneuploidy, t(4;14), t(14;16), t(14;20), and del(17p) are considered as negative prognostic indicator. Therefore, several studies for association between antigenic expression and cytogenetic abnormality have been conducted (11, 12). We analyzed the plasma cell myeloma case and investigated association of

antigen expression pattern with cytogenetic abnormalities and clinical stage.

METHODS AND MATERIALS

Patient Selection and Basic Characteristics

We reviewed the plasma cell myeloma cases newly diagnosed at our hospital from January 2013 to July 2013. The

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TABLE 1. Characteristics of Antibody and Optical Emission Flowcytometer Laser Line/Filters Used in Flowcytometry

Antibody	Fluorochrome	Clone	Manufacturer	Laser line (nm)	Emission filter (nm)
Anti-Kappa, cytoplasmic	PE-Cy7	G20-193	BDIS (San Jose, CA)	561	780/60
Anti-Lambda, cytoplasmic	FITC	1-155-2	BDIS (San Jose, CA)	488	530/30
CD45	PerCP	2D1	BDIS (San Jose, CA)	488	695/40
CD38	FITC	HIT2	BDIS (San Jose, CA)	488	530/30
	APC	HB7	BDIS (San Jose, CA)	633	660/20
CD138	PE	MI15	BDIS (San Jose, CA)	561	575/26
CD117	PE-Cy7	104D2	BDIS (San Jose, CA)	561	780/60
CD274	PE-Cy7	MIH1	BDIS (San Jose, CA)	561	780/60
CD28	PerCP-Cy5.5	L293	BDIS (San Jose, CA)	488	695/40

diagnosis of plasma cell myeloma was based on morphology, immunophenotyping, protein electrophoresis, and manifestations of end organ damage outlined in the 2008 WHO classification (13). We selected the 68 cases of symptomatic or smoldering plasma cell myeloma initially diagnosed at our hospital from January 2013 to July 2013. The basic characteristics such as age, sex, hemoglobin, serum albumin, and serum calcium and serum β 2-microglobulin levels were analyzed. According to the ISS for multiple myeloma (10), we classified the patients as Stage I, II, III.

Conventional Cytogenetics and FISH Study

The conventional cytogenetic analysis was performed using standard G-banding short-term culture. Chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature 2009. Hypodiploidy was defined as ≤ 44 chromosome number and hyperdiploidy was defined as ≥ 47 and < 75 chromosome number. A FISH study was performed using Abbott Molecular probe (Abbott Molecular Inc., Des Plaines, IL) for LSI D13S319, LSI IGH/FGFR3, LSI p53.

Immunophenotyping of Plasma Cell

Red blood cells were lysed using FACS Brand Lysing Solution (Becton Dickinson, San Jose, CA). Mononuclear cells were separated using Ficoll-Hypaque. Washed mononuclear cells were suspended with phosphate buffered saline, resulting in 1×10^7 cells/ml. The flow cytometer was FACSCantoII (Becton Dickinson, San Jose, CA) and analyzing software was Kaluza (Beckman Coulter, Brea, CA).

Immunophenotyping was performed using the following panel of antibody: cytoplasmic-lambda/cytoplasmic-kappa/CD138/CD38/CD19, CD38/CD138/CD274/CD45/CD56, CD38/CD138/CD117/CD28. The characteristics of antibodies, laser lines, and optical emission filters were summarized in Table 1. Compensation matrix was calculated using BD CompBead particles (Becton

Dickinson, San Jose, CA) and the compensation setup tool in BD FACSDiva software.

Neoplastic plasma cells were defined as the CD38 and CD138 positive population with light chain restriction (kappa/lamba $> 4:1$ or < 0.5) (14). The positive cut-off levels of antigen expression was 20% compared to antibody-fluorochrome matched isotype controls. Minimum of 50 000 cells (typically 100,000) was acquired in order to reach sufficient sensitivity. Primary gating of plasma cells were illustrated in Figure 1.

Association of Antigen Expression and Myeloma Stage According to the ISS for Multiple Myeloma

We statistically analyzed the association of antigen expression and ploidy status (hypodiploidy or hyperdiploidy) or FISH results. The association between myeloma stage according to the ISS for multiple myeloma (10) and antigen expression were also analyzed statistically.

Categorical data were compared using the chi-square test or Fisher's exact test. Linear-by-linear association was used to evaluate trends in myeloma stage according to the expression of antigen. P -value < 0.05 was considered statistically significant. Statistical analysis was done with PASW Statistics 20.0 (IBM, Armonk, New York).

RESULTS

Basic Characteristics and Immunophenotyping Results

A total of 68 cases of symptomatic/smoldering plasma cell myeloma were enrolled in this study. The basic characteristics of enrolled cases were summarized in Table 2. Mean age was 63 years; percentage of male was 57.4%. IgG type was most prevalent (45.6%). The percentage of myeloma stage I, II, and III was 32.4% (22/68), 39.7% (27/68), and 27.9% (19/68), respectively. The positive expression of CD19, CD28, CD45, CD56, CD117, and CD274 were 8.8%, 50.0%, 50.0%, 75.0%, 39.7%, and 2.9%, respectively, (Table 3).

Fig. 1. The primary gate of plasma cells (P) and antigen expression patterns of plasma cell. The neoplastic plasma cells were defined as the CD38 and CD138 positive population with light chain restriction. The Cut-off level for positive expression was 20%. Examples of myeloma cases with CD19–, CD56+, CD28–, 117–, CD274–, and CD45– were illustrated.

TABLE 2. Characteristics of 68 Patients With Plasma Cell Myeloma

Characteristics	
Mean age $\pm$ SD (years)	63 $\pm$ 9
Sex	
Male	39 (57.4%)
Female	29 (42.6%)
Type of immunoglobulin	
IgG	31 (45.6%)
IgA	11 (17.6%)
IgM	0 (0.0%)
IgD	5 (7.4%)
light chain only	20 (29.4%)
Kappa	37 (54.4%)
Lambda	31 (45.6%)
Mean hemoglobin $\pm$ SD, g/dL	10.9 $\pm$ 2.1
Mean serum calcium $\pm$ SD, mg/dL	9.5 $\pm$ 1.7
Mean serum albumin $\pm$ SD, g/dL	3.6 $\pm$ 0.7
Mean serum β 2-microglobulin $\pm$ SD, mg/L	4.9 $\pm$ 4.1
International Staging System for myeloma (ISS) ^a (%)	
I	22 (32.4%)
II	27 (39.7%)
III	19 (27.9%)

^aPublished by the International Myeloma Working Group in 2005.

Association of Antigen Expression and Cytogenetic Abnormality

Total 65 of 68 cases were available for conventional chromosomal results. Hypodiploidy cases were 8 of 65 cases (12.3%). Hyperdiploidy cases were 14 of 65

cases (21.5%). Total 64 of 68 cases were available for FISH results. The prevalence of del(13q) or aneuploidy, IGH/FGFR and del(17p13) was 31.3% (20/64), 9.4% (6/64), and 10.1% (7/64), respectively.

CD117 negative cases showed more prevalent of hypodiploidy (8/38, 21.1%) than CD117 positive cases (0/27, 0%) ($P = 0.017$, Table 4). CD45 negative cases were associated with del(13q) or aneuploidy ($P = 0.003$) and del(17p13, $P = 0.011$, Table 5).

Association of Antigen Expression and Myeloma Stage According to the ISS for Multiple Myeloma

We statistically analyzed association between antigen expression and myeloma stage using P for linear-by-linear association (Table 6). CD19/CD28/56/274 expression and myeloma stage were not statistically significant. The percentage of stage I, II, and III in CD45 positive cases were 41.1% (14/34), 47.1% (16/34), and 11.8% (4/34), respectively. The prevalence of stage I, II, and III in CD45 negative cases were 23.5% (8/34), 32.3% (11/34), and 44.1% (15/34), respectively. The CD45 negative cases were significantly associated with advanced stage ($P = 0.012$).

The prevalence of stage I, II, and III in CD117 positive cases were 37.0% (10/27), 59.3% (16/27), and 3.7% (1/27), respectively. The prevalence of stage I, II, and III in CD117 negative cases were 29.3% (12/41), 26.8% (11/41), and 43.9% (18/41), respectively. The CD117

TABLE 3. Antigen Expression Pattern of Plasma Cell Myeloma (*n* = 68)

Antigen	Normal expression ^a	Abnormal expression ^a	Current study
CD19 ^a	Positive (>70%)	Negative (95%)	Negative, 62 (91.2%)
CD28 ^a	Negative (<15%)	Positive (15–45%)	Positive, 34 (50.0%)
CD45 ^b	Positive (69.5%)	Negative (31.5%)	Negative, 34 (50.0%)
CD56 ^a	Negative (<15%)	Positive (75%)	Positive, 51 (75.0%)
CD117 ^a	Negative (0%)	Positive (30%)	Positive, 27 (39.7%)
CD274 ^c	Negative (0.1–2.7%)	Positive (0–92%)	Positive, 2 (2.9%)

^aReference No. 3.^bReference No. 5.^cReference No. 28.

negative cases associated with advanced stage statistically ($P = 0.016$).

DISCUSSION

In this study, we analyzed the association between antigen expression pattern and cytogenetic abnormalities/myeloma stage. The prevalence of CD19 negative cases was slightly lower than other studies (95% vs. 91.2%). The prevalence of CD117 positive cases was higher than other study (30% vs. 39.7%). The prevalence of antigen expression of CD28, CD45, CD56, and CD274 was similar to previous results. Deletion 13q or aneuploidy, IGH/FGFR, and deletion 17p are considered as poor prognostic subgroup in myeloma (15). FISH can detect these cytogenetic abnormality more sensitively than conventional cytogenetics (16). Therefore, we reviewed the FISH results and conventional cytogenetics results simultaneously.

CD117 is hematopoietic growth factor and are not found in normal plasma cell. About 30% of plasma cell myeloma expresses the CD117. In one study, CD117 positive case were showed better progression-free survival (median = 44 months) than CD117 negative cases (median =

33 months) (4). And CD117 negative cases were associated with del(13q), t(14;16), t(11;14) or translocation involving IGH gene. Another study has shown that CD117 negative cases were higher in nonhyperdiploidy group (17). In this study, we found that CD117 negative cases were associated with hypodiploidy and advanced stage. Therefore, these results indicate that CD117 negative cases belong to the potential poor prognosis group and CD117 can be used as a prognostic indicator. European Myeloma Network also recommends the use of CD117 as prognostic indicator (3). CD28 is also recommended by European Myeloma Network as prognostic indicator. CD28 is expressed normally on T-cell, act as costimulator. About 15–45% of plasma cell myeloma was found to express the CD28 and our results were 50%. Previous study has shown that CD28 positive cases were higher in non-hyperdiploidy group than hyperdiploidy group (17). And CD28 positive cases were associated with t(14;16) and del(17p) (4). These results indicate the prognostic value of CD28. However, we did not find an association between CD28 expression and cytogenetic abnormality or myeloma stage.

CD45 is a leukocyte common antigen and about 30% of plasma cell myeloma has shown to be lacked or low expression of CD45. The CD45 negative cases were 50%

TABLE 4. Association of Antigen Expression Patterns and Chromosomal Ploidy Status (*n* = 65)

		Hypodiploidy	Nonhypodiploidy	<i>P</i> -value	Hyperdiploidy	Non-hyperdiploidy	<i>P</i> -value
CD19	+	1	5	0.561	1	5	1.000
	–	7	52		13	46	
CD28	+	3	28	0.711	4	27	0.137
	–	5	29		10	24	
CD45	+	2	32	0.138	5	29	0.229
	–	6	25		9	22	
CD56	+	6	42	1.000	12	36	0.323
	–	3	15		2	15	
CD117	+	0	27	0.017 ^a	8	19	0.227
	–	8	30		6	32	
CD274	+	0	2	1.000	0	2	1.000
	–	8	55		14	49	

^aCD117 negative cases were associated with hypodiploidy.

TABLE 5. Association of Antigen Expression Patterns and Cytogenetic Abnormality

		Deletion 13 or aneuploidy ^a			IGH/FGFR ^b			del (17p) ^b		
		+	–	<i>P</i> -value	+	–	<i>P</i> -value	+	–	<i>P</i> -value
CD19	+	0	6	0.583	0	6	1.000	1	5	0.516
	–	12	47		6	52		6	52	
CD28	+	3	28	0.081	4	26	0.407	4	26	0.697
	–	9	25		2	32		3	31	
CD45	+	1	33	0.001 ^c	1	30	0.198	0	31	0.011 ^c
	–	11	20		5	28		7	26	
CD56	+	8	40	0.717	6	42	0.323	5	43	1.000
	–	4	13		0	16		2	14	
CD117	+	2	25	0.102	1	24	0.391	2	23	0.695
	–	10	28		5	34		5	34	
CD274	+	0	2	0.494	0	2	1.000	0	2	1.000
	–	12	51		6	56		7	55	

^aMetaphase analysis.^bFISH (fluorescence in situ hybridization) study.^cCD45-negative cases were significantly associated with deletion 13 or aneuploidy by metaphase analysis and del(17p) by FISH.

in our study. The clinical significance of CD45 positive or negative myeloma is unclear. However, in one study reported that the CD45 positive plasma cells were associated with delayed disease progression (18). In some previous study, CD45 negative cases were associated with short overall survival (5, 19). In our results, CD45 negative cases were associated with advanced stage, del(13q) or aneuploidy and del(17p).

CD56 can be found in about 75% of plasma cell myeloma. In one study, CD56 negative case were associated with t(11;14) (20). Several studies have shown that CD56 is associated with biological risk factor and poor prognosis (21, 22). However, some studies reported that CD56 was not associated with adverse prognosis or genetic abnormality in high-dose chemotherapy group or autologous stem cell transplant group (20, 23). In this

study, we did not found association between CD56 and cytogenetic abnormality or myeloma stage.

We did not found any association between CD19/CD274 and cytogenetic or myeloma stage. Lack of CD19 expression is characteristic of plasma cell myeloma (3). The clinical significance of CD19 is diagnostic value rather than prognostic indicator (2, 3, 24). CD274 was reported to be found in subpopulation of plasma cells in myeloma (25–28). The clinical significance of CD274 as a prognostic indicator is unclear yet although therapeutic implication of blockade of CD274 has been suggested in a few studies (25–28).

In conclusion, we analyzed the association between antigen expression and cytogenetic or biological stage using newly diagnosed symptomatic/smoldering myeloma. We found the association between CD45 negative cases

TABLE 6. Association of Antigen Expression Patterns and International Staging System for Myeloma (ISS)^a (*n* = 68)

		Stage I	Stage II	Stage III	<i>P</i> -value ^c
CD19	+	2	2	2	1.000
	–	20	25	17	
CD28	+	14	14	6	0.061
	–	8	13	13	
CD45	+	14	16	4	0.012 ^b
	–	8	11	15	
CD56	+	16	21	14	1.000
	–	6	6	6	
CD117	+	10	16	1	0.016 ^b
	–	12	11	18	
CD274	+	0	2	0	–
	–	22	25	19	

^aPublished by the International Myeloma Working Group in 2005.^bCD45, CD117 negative cases were associated with advanced stage of ISS.^c*P*-value for linear-by-linear association of stage according to the expression of antigen.

and adverse cytogenetic abnormalities (deletion 13q or aneuploidy, deletion 17p13) or myeloma stage. And CD117 negative cases were associated with hypodiploidy and myeloma stage. Therefore, adverse cytogenetic abnormality can be predicted using antigen expression pattern.

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