

Flow cytometry-based immunophenotypic analysis of primary systemic light chain amyloidosis

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Received April 5, 2016; Accepted December 20, 2016

DOI: 10.3892/ol.2017.5767

Abstract. Using flow cytometry, the present study aimed to investigate the immunophenotypic characteristics of malignant plasma cells (PCs) in the bone marrow of patients with primary systemic light chain amyloidosis (AL) compared with the characteristics of patients with multiple myeloma (MM). Flow cytometric results of 51 patients with AL and 150 patients with MM were reviewed. The proportion of total bone marrow PCs in the patients with AL was significantly lower than that in the patients with MM, 1.35% (0.3-9.5%) vs. 9.7% (0.4-75.7%); (P<0.001). The cells in 24/51 patients with AL comprised two distinct populations: Normal PCs and malignant PCs, whereas only 11/150 patients with MM exhibited two populations. Patients with AL exhibited an increased cluster of differentiation (CD)19 expression compared with patients with MM, 4.5% (0.1-80.9%) vs. 1.6% (0.1-33.6%); P<0.001 and reduced CD138, with 90.8% (30.4-99.9%) vs. 95.0% (40.7-100%); P=0.006 and CD56, with 61.0% (1.5-99.2%) vs. 98.3% (0.1-100%); P<0.001 expression in malignant PCs. Light chain restriction was identified in all patients with AL, and the proportion of λ light chain restriction was 72.5%. The immunophenotypic characteristics of patients with AL demonstrated by flow cytometry were different compared with the characteristics of patients with MM in the number and composition of plasma cells, and the intracellular and extracellular expression of antigens. These differences may be associated with a less malignant phenotype of clonal PCs in AL than MM.

Introduction

Primary systemic light chain amyloidosis (AL) is a clonal plasma cell (PC) disorder whereby a monoclonal population of

bone marrow PCs produces misfolded light chains that leads to multiple organ dysfunction (1). Although the toxic light chain repertoire has been intensively studied in AL, data concerning pathological clonal PCs, particularly their immunophenotypic features remains limited. In 2003, Shimojima *et al* (2) classified PCs into immature, intermediate and mature stages according to their expression of cluster of differentiation (CD)45, CD49e and MPC-1 antigen (MPC-1), and revealed that the majority of PCs in patients with AL were of the intermediate subtype (MPC-1+CD45-CD49e-), whilst the majority of the PCs in patients with monoclonal gammopathy of unknown significance (MGUS) were mature (MPC-1+CD45+CD49e-). Paiva *et al* (3) summarized the immunophenotypes of the malignant PCs in 35 patients with AL: 100% of the patients were CD38+, 97% of the patients were CD45dim/-, 91% of the patients were CD19- and 59% of the patients were CD56+. The authors proposed that the majority of malignant PC immunophenotypes in patients with AL overlap with the immunophenotypes in patients with multiple myeloma (MM). Previously, a study from China directly compared the immunophenotypic characteristics between 51 patients with AL and 21 patients with MM (4). The authors demonstrated that the two diseases shared similar immunophenotypes, except that the CD117+ rate in patients with AL was higher than that in patients with MM.

Thus far, there have been no well-defined flow cytometry (FCM) immunophenotypic profiles of AL. Therefore, the present study conducted a retrospective analysis of the flow cytometric immunophenotypes in patients with AL and MM who were diagnosed for the first time in the First Affiliated Hospital, Sun Yat-Sen University (Guangzhou, China), to explore the immunological characteristics of amyloidogenic clonal PCs.

Materials and methods

Subjects. The present study included 51 patients with AL that were diagnosed in the First Affiliated Hospital, Sun Yat-Sen University (Guangzhou, China), between November 2008 and March 2015 as the study group. The baseline clinical characteristics are summarized in Table I. All patients with AL underwent biopsy of the affected tissue, and the presence of amyloidosis was confirmed by Congo red staining and/or

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Key words: primary systemic light chain amyloidosis, multiple myeloma, flow cytometry, immunophenotype

by electron microscopy. Additional immunohistochemistry with commercially available monoclonal antibodies directed against λ -light chain [ready-to-use (RTU); cat. no. A0193] and κ -light chain (RTU; cat. no. IR506) (both Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) suggested that all fibrillar amyloids were composed of immunoglobulin light chains (5). An EnVision™ FLEX+ Mouse LINKER system (Dako; Agilent Technologies, Inc.) was used to visualize the antigens (cat. no. K800221). A total of 150 newly diagnosed patients with MM were designated as the MM group, and all patients in this group met the 2011 International Myeloma Working Group diagnostic criteria (6). A separate non-plasma cell neoplasm (PCN) control group was also established and was referred to as the control group. This group consisted of 30 patients where the presence of PCN was ruled out by flow cytometric immunophenotypic examination and immunofixation electrophoresis. The 30 patients included 7 patients with primary glomerulonephritis, 4 with acute or chronic renal failure, 5 with respiratory infections, 2 with rheumatoid arthritis, 2 with systemic lupus erythematosus, 2 with Sjogren's syndrome, 1 with aplastic anemia, 1 with lymphoma, 1 with gastric cancer bone metastasis, and 5 with osteoporosis. No statistically significant difference was observed between gender composition ($P=0.73$) and age ($P=0.07$) among the 3 groups, as demonstrated in Table II. The study was approved by the Investigational Review Board at the First Affiliated Hospital, Sun Yat-Sen University (Guangzhou, China), and written informed consent was obtained from all patients involved.

Morphological examination of bone marrow cells. Morphological evaluation was performed independently without knowledge of the flow cytometry findings. Each case was reviewed at least by one hematopathologist, and the diagnosis of cases that fulfilled the initial inclusion criteria was confirmed by another hematopathologist for this study. For each case, Wright-Giemsa stained BM aspirate smears were evaluated. A cell count of 500 was performed based on examination of multiple fields of BM aspirate smears via light microscopy. Cases with inadequate quality of BM smears were not included in this study.

Specimen collection, preparation, immunofluorescence labeling, and flow cytometry analysis. BM aspirate samples were collected in EDTA-anticoagulant and processed within 24 h of collection. Following incubation with monoclonal antibodies for 20 min at 20°C, erythrocytes were lysed with ammonium chloride (PharmLyse™; BD Biosciences, San Jose, CA, USA) at room temperature for 10 min using a standard lyse/wash technique (7). The antibody information is illustrated in Table III. Samples were acquired on fluorescence FACSCanto instrument (BD Biosciences). A minimum of 100,000 events were acquired. In certain cases with a low number of PC cells, $\leq 500,000$ events were acquired to obtain ≥ 100 cells in the PC gate in all tubes. Data were analyzed using FACSDiva software (version 8.0.1; BD Biosciences). Analysis of the surface and cytoplasmic markers of the plasma cells was performed using a 6-color panel of RTU antibodies and isotype controls conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll, PE-cyanine 7 (PE-Cy7), allophycocyanin (APC) and/or

Table I. Baseline characteristics of patients with light-chain amyloidosis.

Characteristic	Value [median (min-max) or no. (%)]
Serum albumin, g/dl	2.7 (1.4-4.2)
Serum creatinine, μ mol/l	144.42 (33-713)
Serum alkaline phosphatase, IU/l	107.14 (36-552)
β 2-Microglobulin, mg/l	5.4 (1.1-38.5)
Urine total protein, g/24 h	4.74 (0.05-16.62)
Serum M-component, g/l	6.8 (0-23.5)
Urine M-component, g/24 h	0.14 (0-3.37)
Serum cardiac troponin T, ng/ml	0.26 (0.0-5.03)
NT-ProBNP, pg/ml	10,304 (6-76,874)
Number of organs involved	
2	27/51 (52.9)
≥ 3	24/51 (47.1)
Kidney involvement	41/51 (80.4)
Heart involvement	32/44 (72.7)
Liver involvement	8/47 (17.0)
Peripheral nerve involvement	21/25 (84.0)

NT-ProBNP, N-terminal pro B-type natriuretic peptide.

APC-Cy7. The antibody panel included: i) Anti-CD38, -CD56, -CD19, -CD20, -CD138 and -CD45; ii) anti-cytoplasmic (c) κ , - (c) λ , -CD56, -CD138, -CD38 and -CD45; and iii) anti-CD38, -CD54, -CD138 and -CD45 (all RTU; Table III).

CD38/CD138/CD45/side scatter (SSC) was used as the gate to identify the subpopulations of PCs in the bone marrow. The levels of expression of CD19 and CD56 on the cell surface and the cytoplasmic expression of light chains was analyzed to distinguish between benign and malignant PCs (8,9). Cell surface expression levels $>20\%$ for CD19, CD20, CD56, CD54 or CD138 were defined as positive.

Statistical analysis. Unless noted specifically, all data in the present study are expressed as the median (range). Normally distributed data were compared using unpaired Student's t-test or analysis of variance. Data that were not normally distributed were tested using the Mann-Whitney U test and ratios were compared using the χ^2 test. $P<0.05$ was considered to indicate a statistically significant difference. Receiver operating characteristic (ROC) curves were created to identify cut-off values for the flow cytometry indexes to differentiate patients with AL and MM. Data collection, management and statistical analysis were conducted using SPSS (version 17.0; SPSS, Inc., Chicago, IL, USA).

Results

Patients with AL exhibited lower bone marrow PC proportions compared with patients with MM. Whether measured

Table II. Patient demographics.

Variable	AL group	MM group	Control group	P-value
Male (%)	32/50 (62.7)	100/150 (66.7)	18/30 (60.0)	0.73
Age [Median (min-max)]	58 (33-79)	56 (31-75)	52 (27-77)	0.07

AL, primary systemic light chain amyloidosis; MM, multiple myeloma.

Table III. Antibodies used for flow cytometry.

Antibodies	Cat. no.	Concentration (μ l/100 μ l)	Manufacturer
Anti-CD38-FITC/CD56-PE/ CD19-PerCP-Cy5.5	341132	20	BD Biosciences, San Jose, CA, USA
Anti-CD20-PE-Cy7	335793	5	BD Biosciences
Anti-CD38 (HB-7)-APC	345807	5	BD Biosciences
Anti-CD54-PE	555511	20	BD Biosciences
Anti-CD138-PerCP-Cy5.5	341087	20	BD Biosciences
Anti- κ FITC/ λ -PE	349516	20	BD Biosciences
Anti-CD45-APC	340943	5	BD Biosciences

CD, cluster of differentiation; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll; Cy, cyanine; APC, allophycocyanin.

by cell morphology or FCM, the majority of patients with AL exhibited bone marrow PC proportions of <10%, as demonstrated in Table IV. Patients with AL exhibited a lower total PC proportion by FCM compared with patients with MM, 1.35% (0.3-9.5%) vs. 9.7% (0.4-75.7%; $P < 0.001$). The optimal cut-off value of the total PC proportion by FCM to distinguish between the two was 4.2% (area under the ROC curve=0.88; $P < 0.001$). The sensitivity was 92.2% and the specificity was 72.7%.

A total of two populations of malignant and normal PCs were detected more frequently in AL compared with MM. Amongst the 51 patients with AL, benign and malignant PCs were detected in the bone marrow samples from 24 patients, whereas the two populations of PCs were detected in only 11/150 patients with MM, and the difference was statistically significant (47.1 vs. 7.3%; $P < 0.001$), as demonstrated in Fig. 1. The 11 patients with MM exhibiting two populations of PCs demonstrated higher malignant PC proportions among the total PCs compared with the 24 patients with AL who exhibited two populations of PCs, 90.67% (50.0-98.36%) vs. 76.39% (33.33-91.67%; $P = 0.008$), as illustrated in Fig. 2.

Malignant PCs were slightly smaller in patients with AL than in patients with MM. The size and granularity of the PCs were defined by forward scatter and SSC, respectively. Malignant PCs were smaller in patients with AL compared with patients with MM ($P = 0.009$), as demonstrated in Table V and Fig. 3, although the difference in cell granularity was not statistically significant ($P = 0.473$). AL malignant PCs were larger

($P = 0.021$) but showed less granularity ($P = 0.014$) compared with the control PC group.

Patients with AL and MM differed in malignant PC surface antigen expression. The expression patterns of the surface antigens in malignant bone marrow PCs from patients with AL and MM and those in the PCs from the control group are illustrated in Table VI. Bright expression of CD38 was detected in all patients. CD19 expression on the surface of malignant PCs increased in patients with AL, while CD138 and CD56 expression levels decreased compared with patients with MM.

CD138 expression levels on the surface of malignant PCs were lower in AL patients than in MM patients. The CD138 expression level was lower in patients with AL compared with patients with MM, 90.8% (30.4-99.9%) vs. 95.0% (40.7-100%; $P = 0.006$), but was higher compared with the control group, 81.0% (29.7-95.2%; $P = 0.024$). The ROC curve showed that the best critical point of CD138 expression to differentiate between AL and MM was 94.25% (area under the ROC curve=0.63; $P = 0.003$), with a sensitivity of 72.5% and a specificity of 53.3%.

CD19 expression levels on the surface of malignant PCs were higher in patients with AL compared with patients with MM. Of the 51 patients with AL, 7 exhibited positive CD19 expression whereas only 4/150 patients with MM were CD19+, a difference that was statistically significant, 13.7 vs. 2.7% ($P = 0.007$). The CD19 expression level was higher in patients with AL compared with patients with MM, 4.5% (0.1-80.9%)

Table IV. Bone marrow plasma cell counts assessed by morphology and FCM.

Variable	AL group	MM group	Control group	P-value
PCs by morphology (%)	4 (0.5-16) ^{a,b}	24 (3-85)	1.5 (0.5-12)	<0.01
PCs by FCM (%)	1.35 (0.3-9.5) ^{a,b}	9.7 (0.4-75.7) ^a	0.7 (0.1-4.6) ^b	<0.01
M-PCs by FCM (%)	1.10 (0.14-9.5) ^{a,b}	9.7 (0.24-75.7) ^a		<0.01

^aP<0.05 vs. control group. ^bP<0.001 vs. MM group. Data are expressed as the median (range). FCM, flow cytometry; PCs, plasma cells; M-PCs, monoclonal PCs; AL, primary systemic light chain amyloidosis; MM, multiple myeloma.

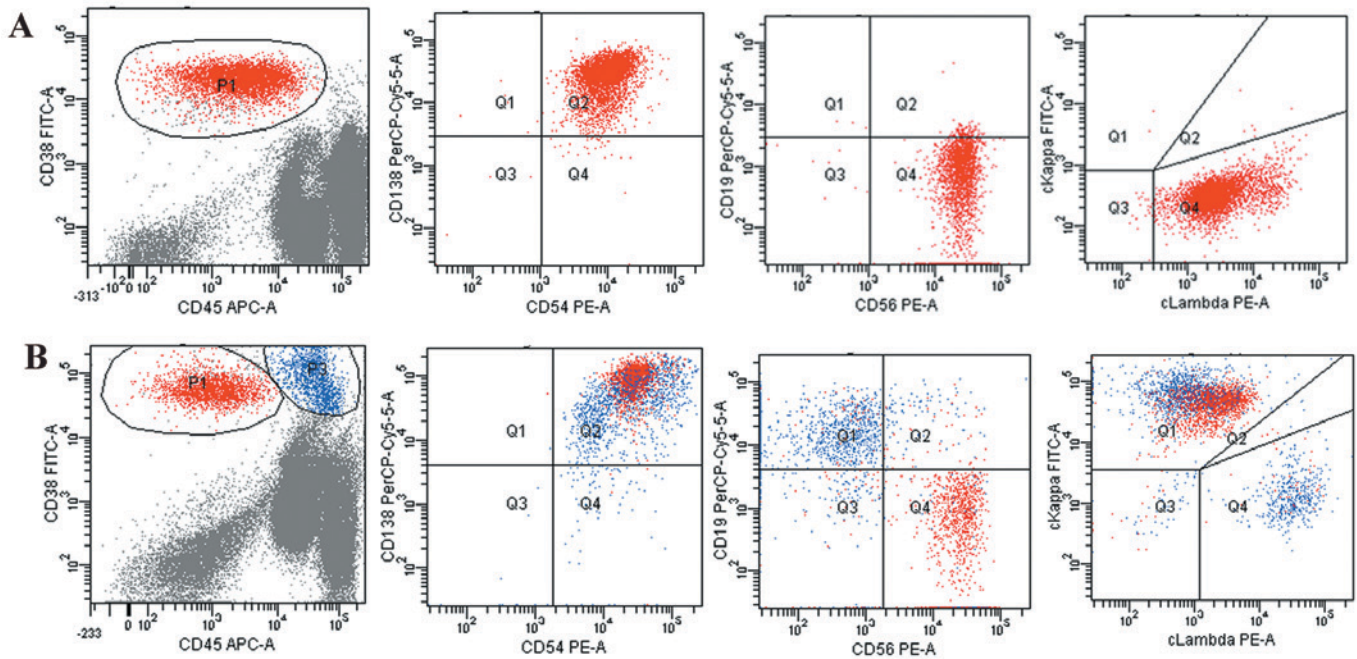


Figure 1. Examples of typical immunophenotypes and clonality assessments of PCs in bone marrow samples from the AL and MM groups. (A) Immunophenotypes of malignant bone marrow PCs in one patient with MM with the immunophenotype CD38+CD45-/dimCD138+CD19-CD56+ and an abnormal cytoplasmic κ/λ light chain ratio. (B) Flow cytometry results from one patient with AL. Malignant PC populations without normal PCs were detected in the majority of bone marrow samples from patients with MM (139/150; 92.7%). Whereas two distinct populations, normal PCs and malignant PCs, were detected in ~50% of patients with AL (24/51; 47.1%). CD, cluster of differentiation; APC-A, allophycocyanin-A; PE-A, phycoerythrin-A; FITC-A, fluorescein isothiocyanate; AL, primary systemic light chain amyloidosis; MM, multiple myeloma; PC, clonal plasma cell; P1, malignant plasma cells, with the immunophenotype CD38+CD45-/dimCD138+CD19-CD56+ and an abnormal cytoplasmic κ/λ light chain ratio; P3, normal plasma cells with the immunophenotype CD38+CD45+CD138+CD19+CD56- and a cytoplasmic κ/λ ratio of 1.98.

vs. 1.6% (0.1-33.6%; P<0.001). The majority of the control group patients, 93.3%, exhibited CD19 expression on their PC surfaces and demonstrated high expression levels, 71.8% (0.4-90.3%). The ROC curve showed that the best critical point of CD19 expression for the distinction between the AL and MM groups was 2.5% (area under the ROC curve=0.74; P<0.001), with a sensitivity of 72.5% and a specificity of 67.3%.

The CD56 expression levels on the surface of malignant PCs were lower in patients with AL compared with patients with MM. The CD56+ expression rate on the surface of malignant PCs was lower in patients with AL, 58.8%, compared with patients with MM, 76.7% (P=0.014). In addition, the CD56 expression level was significantly lower in patients with AL, 61.0% (1.5-99.2%) compared with patients with MM, 98.3% (0.1-100%; P<0.001). The ROC curve demonstrated that the

best critical point of CD56 expression to differentiate between the two groups was 95.8% (area under the ROC curve=0.768; P<0.001), with a sensitivity of 94.1% and a specificity of 59.3%.

Ratio of patients with predominant λ light chain in the cytoplasm of malignant PCs was higher in the AL group compared with the MM group. The cytoplasmic κ and λ light chain ratio was abnormal in the malignant bone marrow PCs from patients with AL and MM. The proportion of λ light chain restriction in patients with AL was 72.5%, which was higher compared with the proportion from patients with MM, 72.5 vs. 36.7% (P<0.001).

Discussion

Although AL and MM exhibit different biological behaviors and clinical phenotypes, it has been demonstrated that the

Table V. Comparison of the granularity and size of clonal PCs from AL and MM patients and normal bone marrow PCs from the control group.

Variable	AL group	MM group	Control group
FSC	10,7675 (29,778-190,691) ^{a,b}	129,675 (62,814-216,102)	100,577 (20,944-185,665) ^a
SSC	1,664 (464-5,259) ^b	1,503 (287-4,751) ^b	2,052 (533-3,790)

^aP<0.01 vs. MM group. ^bP<0.05 vs. control group. Data are expressed as the median (range). AL, primary systemic light chain amyloidosis; MM, multiple myeloma; PCs, plasma cells; FSC, forward scatter; SSC, side scatter.

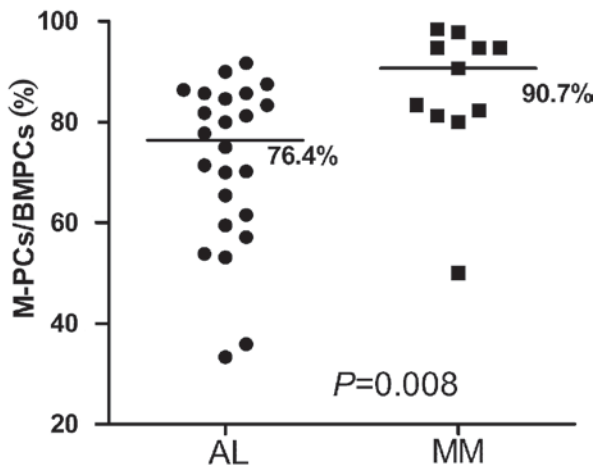


Figure 2. Different ratios of M-PCs/BMPCs between the AL and MM groups. Patients with MM who had two populations of PCs exhibited higher M-PCs proportions among the total PCs compared with patients with AL who had two populations of PCs [90.67% (50.0-98.36%) vs. 76.39% (33.33-91.67%); P=0.008]. PCs, plasma cells; M-PCs, monoclonal plasma cells; BMPCs, bone marrow plasma cells; AL, primary systemic light chain amyloidosis; MM, multiple myeloma.

FCM immunophenotypes of patients with AL overlap with the immunophenotypes of patients with MM (10). AL is hypothesized to exhibit the same disease pattern as MGUS and MM at the cellular level, with AL being a clonal PC disorder with an 'unlucky protein' (11). In the present study, the differences between AL and MM immunophenotypes were systematically compared by FCM. The results indicated that in patients with AL, the proportion of total bone marrow PCs was significantly lower compared with patients with MM and 2 distinct populations, benign cells and malignant cells, were detected in almost half of patients. In addition, the expression of CD19 was higher and the expression of CD56 and CD138 was lower in patients with AL compared with patients with MM.

Consistent with a previous study, the average PC proportion among all bone marrow nucleated cells in the present study was lower in patients with AL compared with patients with MM (12). The proportion of PCs measured by FCM was lower compared with the proportion of PCs measured by the bone marrow smear examinations as; FCM includes bone marrow aspiration, whereby bone grease may be washed away during the specimen preparation, and certain PCs may adhere to the bone grease and the bone marrow aspirate may mix with peripheral blood in the extraction process (13).

In addition to the PC proportion, patients with AL and MM also differed significantly with respect to the composition of PCs. Almost 50% of the bone marrow samples from patients with AL exhibited two types of PC with normal and abnormal immunophenotypes, as detected by flow cytometry. However, PCs exhibiting a normal immunophenotype were only detected in 7.3% of patients with MM. When comparing patients with AL and MM with two populations of PCs, the ratio of malignant PCs in patients with AL was significantly lower compared with patients with MM. In addition to AL, the populations of benign and malignant cells may also be detected in almost all of the patients with MGUS, a pre-malignant condition of MM (14). Furthermore, the proportion of normal PCs amongst the total PCs may assist to distinguish MGUS from MM, therefore screening patients with a high risk of progressing to MM (14,15). It may be suggested that the proliferation index and invasiveness of malignant PCs in AL were more similar to those in MGUS compared with MM.

CD19 is a B cell-specific marker and exhibits almost no expression on the surface of malignant PCs (16). The absence of CD19 in MM PCs suggests the deteriorating progression of tumor cells (17). Mahmoud *et al* (17) increased the cell surface expression levels of CD19 in myeloma cell lines and revealed that tumor cell growth was inhibited and the capacity to generate tumor cells was reduced. The results of the present study indicate that in patients with AL, the CD19 expression in the malignant PCs was significantly higher compared with patients with MM, which may also suggest that AL is less malignant than MM.

CD138, also termed syndecan-1, is one of the specific markers of normal PCs and malignant PCs. CD138 binds to soluble molecules such as epidermal growth factor, fibroblast growth factor and hepatocyte growth factor, and insoluble molecules such as collagen and fibronectin via the terminal heparin sulfate chains. Thus, CD138 mediates cell-cell adhesion and the interaction between cells, and promotes the proliferation and growth of malignant PCs (18). In addition, CD138 is also a PC differentiation antigen. CD138- plasma blasts are PC progenitor cells, which differentiate into CD138+ PC precursors and finally mature into CD138++ PCs that no longer possess the capacity for mitosis and proliferation (19). The present study demonstrated that the CD138 expression level on the surface of malignant PCs in patients with AL was lower compared with patients with MM, suggesting that clonal PCs from patients with AL may differ from the PCs of patients with MM in terms of proliferation, growth and interaction with the bone marrow microenvironment.

Table VI. Immunophenotypic characteristics of clonal PCs from the AL and MM groups and bone marrow plasma cells from the control group presented as proportions of cell subpopulations expressing the analyzed antigen.

	AL group	MM group	Control group	P-value		
				AL vs. MM	AL vs. control	MM vs. control
CD138	90.8% (30.4-99.9%)	95.0% (40.7-100%)	81.0% (29.7-95.2%)	0.006	0.024	<0.001
CD19	4.5% (0.1-80.9%)	1.6% (0.1-33.6%)	71.8% (0.4-90.3%)	<0.001	<0.001	<0.001
CD20	4.7% (0.1-95.5%)	4.3% (0.1-99.6%)	5.8% (1.0-22.0%)	0.40	0.37	0.067
CD54	99.1% (61.2-100%)	98.6% (27.2-100%)	97.9% (70.8-99.9%)	0.13	0.07	0.59
CD56	61.0% (1.5-99.2%)	98.3% (0.1-100%)	7.1% (0.7-41.7%)	<0.001	0.003	<0.001

All cases were CD38+. Data are expressed as the median (range). AL, primary systemic light chain amyloidosis; MM, multiple myeloma; CD, cluster of differentiation.

Table VII. Comparison of our data with major studies that focused on the malignant plasma cell phenotypic profile in AL.

	Present study	Hu <i>et al</i> (4)	Paiva <i>et al</i> (3)	Yoshida <i>et al</i> (22)
Year published		2014	2011	2008
Number of patients	51	51	35	59
M-PCs by FCM (%)	1.10 (0.14-9.5) ^a	3.87 (0.17-9.34) ^a	1.4 (0.1-15.0) ^a	2.16±2.12 ^b
CD45-/dim (%)	98	100	97	-
CD56+ (%)	58.8	78.4	59	-
CD19+ (%)	13.7	11.8	9	-
CD20+ (%)	11.8	13.7	-	-
Others (%)	LCR 100	LCR 60.8, CD117+ 69	-	-

^aData are expressed as the median (range); ^bdata are expressed as the mean ± standard deviation; LCR, light chain restriction; M-PCs, monoclonal plasma cells; FCM, flow cytometry; CD, cluster of differentiation.

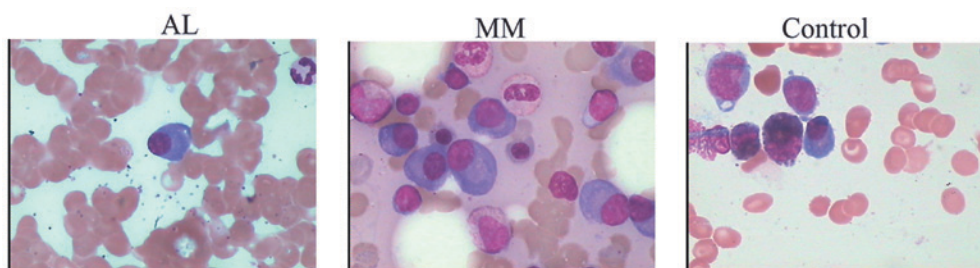


Figure 3. Morphological differences in abnormal plasma cell clones between the AL and MM groups and bone marrow plasma cells from the control group (Wright-Giemsa staining; magnification, x1,000). AL, primary systemic light chain amyloidosis; MM, multiple myeloma.

Moreover, AL may also exhibit lower PC differentiation degrees compared with MM.

The present study also revealed that the level of CD56 expression significantly increased in MM malignant clonal PCs and was relatively low in patients with AL. Ely and Knowles (20) reported that CD56 expression in PCs is associated with osteolytic lesions in patients with myeloma. The CD56 molecule facilitates homeopathic interactions between myeloma cells and osteoblasts, resulting in a decreased generation of bone

matrix and eventually osteolytic bone changes (21). The data from the present study suggest that the interaction between malignant bone marrow PCs and the bone matrix in patients with AL is not as strong as the interaction in MM tumor cells. The data suggest that the two diseases differ in the bone marrow microenvironment surrounding the malignant bone marrow PCs, and that there may be different signaling pathways and interactions affecting the biological behavior of clonal PCs in each disease.

The present study and previous major studies on AL immunophenotypes are summarized in Table VII. The advantage of the present study is that the benign and malignant PCs in the bone marrow from each patient were first distinguished, then the immunophenotypes of the malignant PCs were analyzed. This is particularly important in the patients with AL as the invasiveness of malignant PCs and the ability of malignant PCs to reproduce in patients with AL are not as strong, and the suppression of the normal PCs is relatively weak. A possible reason for the high proportion of PCs in AL obtained by Hu *et al* (4) is that the benign and malignant bone marrow PCs were not distinguished. In addition, unlike the studies of Paiva *et al* (3) and Yoshida *et al* (22), the present study collected the flow cytometry data for patients with AL and MM, and demonstrated the immunophenotypic characteristics of AL malignant PCs clearly with a systematic comparison. The main disadvantages of the present study are that it is a retrospective analysis and the antigens for analysis are limited.

In conclusion, the flow cytometric immunophenotypic characteristics of AL are different compared to the characteristics of MM in the number and the composition of PCs, and the intracellular and extracellular expression of antigens, which may be associated with a less malignant phenotype of clonal PCs in AL compared with MM.

Acknowledgements

The present study was supported by the Program of National Key Clinical Specialties and Science and Technology Planning Project of Guangdong Province, China (grant no. 2014A020212061).

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