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*Original Article*

# Expression of CD19 and CD20 in plasma cells are significantly different between Han and Uygur multiple myeloma patients

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**Abstract:** Objective: To know the relativity between immunophenotypic characteristics and disease staging of 95 Uygur and Han Multiple Myeloma (MM) patients in Xinjiang region. Methods: Four color flow cytometry was used to detect immunophenotype of MM patients. Fisher's exact test and correlational analysis method was used to analyze the difference and relativity of in different races. Results: CD19 and CD20 expressions of plasma cells were significantly different between Uygur and Han MM patients. On stage II and III, positive rates of Uygur and Han MM patients were both showed statistic differences. CD19, CD20 expressions had significant positive correlation with the disease stage in MM patients. Conclusions: Immunophenotypic characteristics and disease staging were different in Han and Uygur MM patients and CD19 and CD20 had relativity with disease staging of MM.

**Keywords:** Multiple myeloma, immunophenotype, disease staging

## Introduction

Multiple myeloma (MM) is a clonal B-cell disorder in which malignant plasma cells (PCs) accumulate in the bone marrow [1, 2]. The great heterogeneity of MM results to the different morbidity in different districts and races [3-5]. It is reported that the morbidity of MM in Austrila, New Zealand, North America and Northern Europe are high, but relatively low in Asia [6]. In addition, the prognosis and survival time varies in different MM patients ranging from several months to a dozen years. Making distinction among MM patients with different prognosis will contribute to select the best treatment, which can both increase the curative effect and reduce the medical costs [7-9]. A conventional diagnosis in MM is based on the laboratory results, such as morphologic features, hematologic features, immunophenotyping, cytogenetics, DNA ploidy, and labeling index-proliferative activity of PCs [10]. Immunophenotypic studies on MM have been performed for more than 15 years, and flow cytometry (FCM) has gradually become important in the diagnosis, prognostication and follow-up of MM [11]. In this study, immunophenotypes of 95 Uygur and Han MM patients were analyzed by FCM, and the relationship between immunophenotype and disease staging was also performed. The results may guide to explore the difference of immunophenotype in different races. Meantime, as a prognostic index with ancillary, the immunophenotypic characteristics of MM also guide clinical diagnosis and treatment.

## Materials and methods

### *Subjects*

Bonemarrow (BM) aspiration samples were obtained from 95 patients with MM from May

**Table 1.** Clinical characteristics of the patients with multiple myeloma (n = 95)

| Characteristics | Number (%) or median (range) |
|---|---|
| Age | Han: 62.5 (44-79); Uygur: 57.5 (39-72) |
| Gender (male: female) | Han: 34:18 (Ratio: 1.89:1); Uygur: (27:16) (1.69:1) |
| Nationality (Han: Uygur) | Han (52): Uygur (43) |
| Myeloma subtype | |
| IgG | Han: 23; Uygur: 20 |
| IgA | Han: 14; Uygur: 12 |
| Light chain | Han: 11; Uygur: 9 |
| Others | Han: 4; Uygur: 2 |
| Disease Stage | |
| I | 19 (20) (Han: Uygur = 13:6) |
| II | 28 (29) (Han: Uygur = 20:8) |
| III | 48 (51) (Han: Uygur = 19:29) |

2012 to May 2014. The details of all patients were shown in **Table 1**.

*FCM*

Multiparaetric FCM immunophenotyping was performed using monoclonal antibodies against CD19, CD20, CD45, CD56, CD117 and HLA-DR. Four antibodies conjugated with FITC, PE, PerCP, and APC (BD Biosciences, USA) were used.

BM samples collected in EDTA anticoagulant were filtered by 300 mesh nylon net to a final adjusted cell count of 4-10×$10^6$/L. 50 μL of BM were incubated with 10 μL each of the monoclonal antibodies for 15 minutes at room temperature in the dark. Then hemolysis solution (BD Biosciences) was used to lyse erythrocytes for 8 minutes at room temperature in the dark. After centrifugation, the cells were washed with phosphate buffered saline (PBS) twice to analyze the membrance antigens in a flow cytometer (FACS Calibur, BD).

For intracellular antigens, after washing with PBS, 50 μL adjusted cell was incubated with 10 μL surface monoclonal antibodies for 10 minutes at room temperature in the dark. Then 50 μL of Fix and Perm reagent A solution was added. 5 minutes of incubation, the cells were lysed by hemolysis solution for 8 minutes at room temperature in the dark. 25 μL of Fix and Perm reagent B solution together with anti-k and anti-l light chain antibodies were added and incubated for 15 minutes. The supernatant was discarded and cells were washed with PBS twice to analyze in a flow cytometer (FACS Calibur, BD).

*Statistical analysis*

All data were analyzed using SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA). Fisher's exact test and non-parametric spearman correlational analysis method were used in this study. $P<0.05$ was considered statistically significant.

## Results

*The immunofluorescence analysis by flow cytometer*

In 95 cases, plasma cells could be sufficiently identified through initial CD38++/CD138++ gating. The overall positive expression rates of CD56, CD117, CD20, CD19, CD45 and HLA-DR were 75.79% (72/95), 38.95% (37/95), 33.68% (32/95), 17.89% (17/95), 27.37% (26/95) and 32.63% (31/95), respectively (**Figures 1A**, **1B**, **2**).

*Immunophenotype differences between Uygur and Han MM patients*

PCs CD19 and CD20 expressions were significantly different between Uygur and Han MM patients, but other immunophenotypes had no difference. Positive rates were different between Hans and Uygurs in different stages of MM. The much higher positive rate were found in Han patients on stage II but reversed in stage III. The differences were statistically significant (**Table 2**).

In the correlation analysis between the immunophenotypes and disease stage in Han MM patients, CD19, CD20 showed the significant positive correlation (**Table 3**). The similar result was shown in Uygur MM patients (**Table 4**).

## Discussion

Multiple myeloma is a common hematological malignancy which is characterized by plasma cell malignancies proliferation and osteolysis destruction. M-protein, bone destruction, anemia, renal function impairment and immune function abnormality are common in clinically

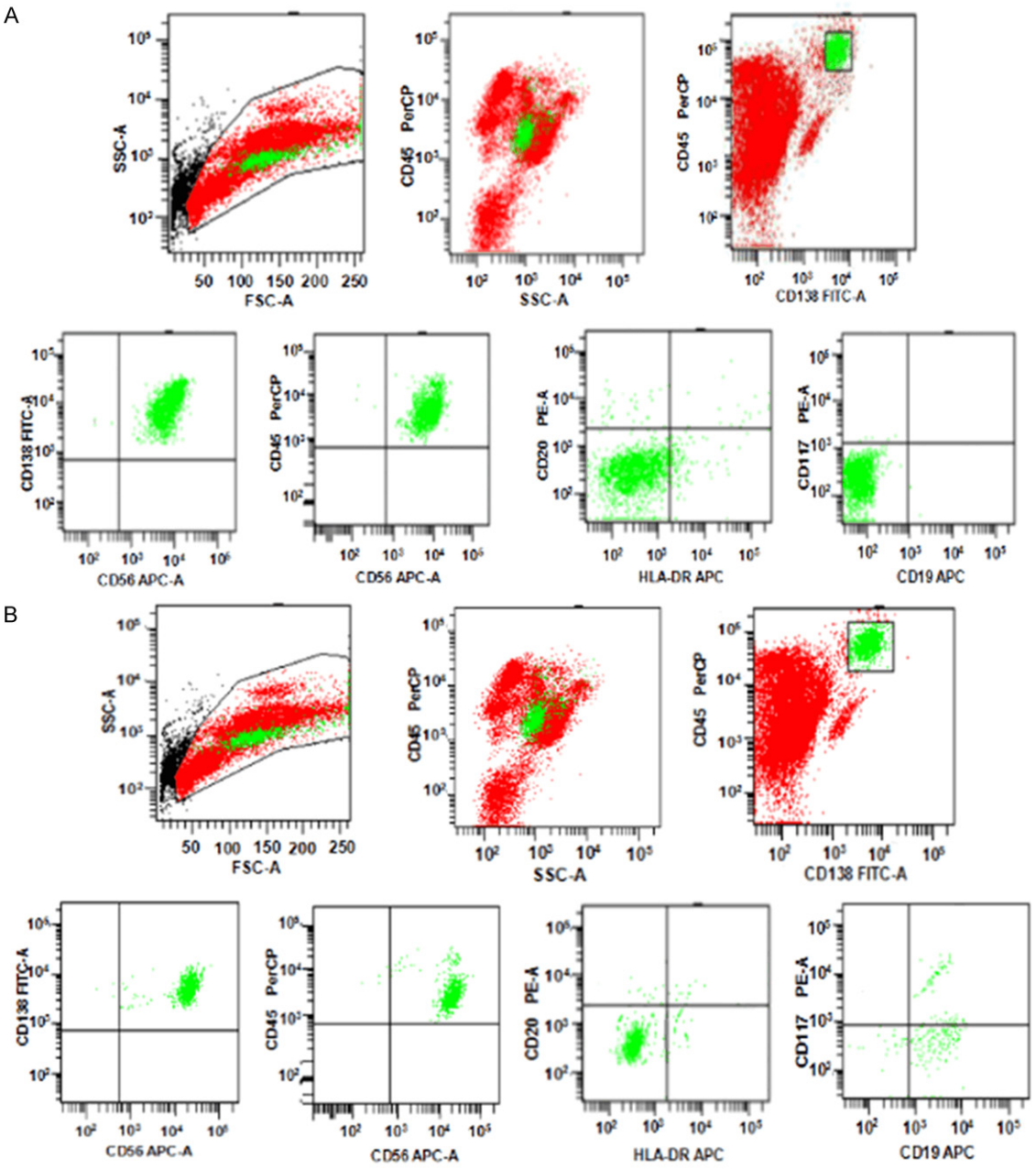


**Figure 1.** Immunofluorescence analysis by flow cytometer. CD38/CD138 and CD45/SSC expression were devised to use as immunofluorescence analysis gate. A. The expression of CD138, CD45, CD56, CD19, CD20, CD117, HLA-DR in Han MM patients; B. The expression of CD138, CD45, CD56, CD19, CD20, CD117, HLA-DR in Uygur MM patients.

[12]. Diagnosis of MM requires different examination of bone marrow, such as PC infiltration in cytomorphology inspection, detection and quantification of monoclonal immunoglobulin in the serum or urine and evidence of organ damage (hypercalcemia, renal insufficiency, anaemia or bone lesions) [13, 14]. Immunophenotyping is a new invaluable tool in the management of hematological malignancies and is increasingly finding an important role in diagnosis and monitoring of plasma cell disorders [15, 16]. Nowadays, it is common to detect immunophenotype by FCM in differentiating diagnosis, estimating prognosis and detecting the minimal residual disease of MM [17-19].

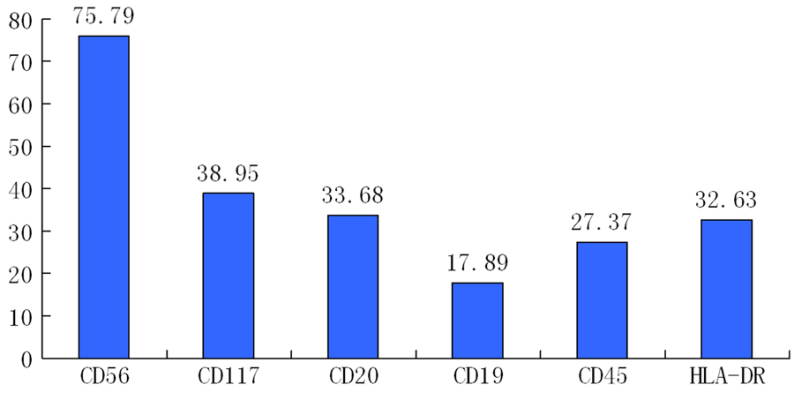


**Figure 2.** The positive rate of immunofluorescence in PCs. Positivity for antigen expression on flow cytometry was defined as staining of >20% of the cells.

**Table 2.** Immunophenotypes and disease stage of Han and Uygur MM patients [n (%)]

| Immunophenotype | Han (n = 52) | Uygur (n = 43) | $X^2$ | P |
|---|---|---|---|---|
| CD56 (n, %) | 36 (69.23) | 36 (83.72) | 2.693 | 0.101 |
| CD117 (n, %) | 22 (42.31) | 15 (34.88) | 0.546 | 0.460 |
| CD19 (n, %) | 5 (9.62) | 12 (27.91) | 5.360 | 0.021* |
| CD20 (n, %) | 24 (46.15) | 8 (18.60) | 6.605 | 0.010* |
| HLA-DR (n, %) | 16 (30.77) | 15 (34.88) | 0.181 | 0.067 |
| CD45 (n, %) | 12 (23.08) | 14 (32.56) | 1.064 | 0.302 |
| Stage (n, %) | | | | |
| Stage I | 13 (25.00) | 6 (13.95) | 1.795 | 0.180 |
| Stage II | 20 (38.46) | 8 (18.60) | 4.465 | 0.035* |
| Stage III | 19 (36.54) | 29 (67.45) | 8.992 | 0.003** |

Note: *$P$<0.05, **$P$<0.01.

**Table 3.** Correlation analysis between the immunophenotypes and disease stage in Han MM patients

| Disease stage | CD56 | | CD117 | | CD19 | | CD20 | | HLA-DR | | CD45 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | NC | P | NC | P | NC | P | NC | P | NC | P | NC |
| Stage I | 8 | 4 | 4 | 8 | 1 | 28 | 2 | 10 | 2 | 10 | 6 | 10 |
| Stage II | 18 | 2 | 10 | 10 | 1 | 12 | 10 | 10 | 6 | 14 | 4 | 12 |
| Stage III | 10 | 10 | 8 | 12 | 3 | 7 | 12 | 8 | 8 | 12 | 2 | 18 |
| *r* | -0.214 | | 0.022 | | 0.296 | | 0.308 | | 0.190 | | -0.272 | |
| *P* | 0.128 | | 0.876 | | 0.033* | | 0.026** | | 0.177 | | 0.052 | |

Note: *$P$<0.05, **$P$<0.01. P: patients, NC: normal control.

A multi-center study reported that malignant plasma cell had different antigen expression to normal plasma cell [20-22]. Immunophenotypes of malignant plasma cell often showed: ① Downregulation of CD19, CD27, CD38, CD4 and CD138; ② Overexpression of CD28, CD33 and CD56; ③ CD20, CD117 and surface immunoglobulin expression are not synchronous. Generally, the immunophenotype of normal plasma cell is CD38str$^+$ D138str$^+$ CD45$^+$ D19$^+$ D56$^-$, but CD38str$^+$ CD138str$^+$ CD45$^-$ CD19$^-$ CD56$^+$ of malignant plasma cell.

Detection results of MM antigen expression varied in different laboratories. As Mateo G's study [23], CD56, CD117, CD20, CD19, and CD45 were detected and the expressions of them accounted for 60%, 32%, 17%, 4% and 27% in 685MM patients respectively. In our study, much higher expression of CD19 was found, maybe it was because they come from the different ethnic groups.

Antigen expression of MM patients not only used to identify abnormal myeloma cells, but also related to the prognosis. It is reported that CD19 and CD20 were the two common antigens related to the worse prognosis.

CD19 is one of glycoproteins, with the relative molecular weight 95×10$^3$. CD19 can active and regulate the growth of B cell, which is an acquired biomarker in early stage of B cell differentiation. Most normal plasma cells express CD19 weakly except for part of subpopulation and abnormal myeloma cells. Luiz A [24] showed that comparing with CD19$^-$ MM patients, CD19$^+$ MM patients had worse prognosis and lower progression-free survival (PFS) and overall survival (OS).

CD20 is a unique characteristic for B lymphocyte surface. It is not expression in normal plasma cells but low expression in 13%-22% of myeloma cells [25]. Expression of CD20$^+$ in MM patients also suggest worse prognosis. As a newly target for MM immunotherapy, CD20 had

**Table 4.** Correlation analysis between the immunophenotypes and disease stage in Uygur MM patients

| Disease stage | CD56 | | CD117 | | CD19 | | CD20 | | HLA-DR | | CD45 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | NC | P | NC | P | NC | P | NC | P | NC | P | NC |
| Stage I | 7 | 1 | 3 | 6 | 2 | 15 | 1 | 15 | 2 | 3 | 2 | 6 |
| Stage II | 15 | 2 | 8 | 10 | 3 | 10 | 2 | 16 | 7 | 9 | 2 | 8 |
| Stage III | 14 | 4 | 4 | 12 | 7 | 6 | 5 | 4 | 6 | 16 | 10 | 15 |
| *r* | -0.123 | | -0.055 | | 0.378 | | 0.406 | | -0.15 | | 0.172 | |
| *P* | 0.431 | | 0.725 | | 0.012* | | 0.007** | | 0.336 | | 0.271 | |

Note: *$P$<0.05, **$P$<0.01. P: patients, NC: normal control.

been concerned widely. Kapoor et al [26] treated CD20$^+$ MM patients with Mabthera, 10% patients showed effectiveness and 50%-57% of the patients could control the illness for 10-27 months.

In our study, CD19$^+$ and CD20$^+$ MM patients in different ethnic groups had the worse disease stages. It indicated that CD19$^+$ and CD20$^+$ were related to the clinical stage of MM. Unbalance of ethnic difference, geographic distribution, economic level, medical condition may cause this difference, the further research need to be performed.

In conclusion, FCM was an effective method to detect immunophenotype of MM, which could provide the basic guideance to the diagnosis, clinical stage, estimating prognosis and treatment of MM.

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## Disclosure of conflict of interest

None.

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