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Role of Flow Cytometric Immunophenotyping in Diagnosis of Multiple Myeloma

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Immunophenotyping has become an invaluable tool in the management of hematological malignancies and is increasingly finding a role in the diagnosis and monitoring of plasma cell disorders. The aim of the study was to provide an accurate diagnosis of multiple myeloma by application of flow cytometry immunophenotyping. A cross-sectional descriptive study was conducted at Department of Clinical Hematology, Yangon General Hospital from September 2015 to August 2016. Multiparametric flow cytometry immunophenotyping was performed using monoclonal antibodies against CD56, CD19, CD138, CD38 and CD45. A total of 40 clinically suspected cases of multiple myeloma were included for the study. Among the 40 cases, 25 cases (62.5%) were diagnosed as multiple myeloma by the positive expression of CD138 or CD38, negative CD19 expression, weak or negative CD45 expression and positive or negative CD56 expression. Age of the patients ranged from 49 years to 89 years. The male to female ratio was 1.1:1. Serum protein electrophoresis and densitometer reading were performed in all cases for detection of monoclonal band and monoclonal protein concentration. Monoclonal band was detected visually and estimation of monoclonal protein was done by densitometer. Among 40 cases, 25 (62.5%) had monoclonal gammopathy. Among these cases, 20 (80%) had monoclonal band in the gamma (γ) region and 5 (20%) had in the beta (β) globulin region. The mean concentration of monoclonal protein was 4.50 g/dl, with a range of 2.28 to 7.95 g/dl. The remaining 15 (37.5%) were not detected monoclonal band. In conclusion, flow cytometry immunophenotyping is useful tool for the diagnosis of multiple myeloma and it should be included as a routine assay in monoclonal gammopathy patients.

Key words: Multiple myeloma, Flow cytometry, Immunophenotyping, Diagnosis

INTRODUCTION

Multiple myeloma is a clonal B-cell disorder in which malignant plasma cells accumulate in the bone marrow, producing lytic lesions, excessive amounts of monoclonal protein in the serum or urine, and evidence of end-organ damage (hypercalcemia, renal insufficiency, anemia, or bone lesions).[1] It accounts for 1% of all malignancies and 10% of hematological malignancies.[2, 3]

Evaluation of multiple myeloma disease is based on a variety of laboratory techniques, including bone marrow morphology and immunophenotyping, analysis of serum and urine M- component and free light chains, hematological and biochemical parameters, cytogenetics, DNA ploidy, and measurement of plasma cell proliferative activity. These investigations are important to support the diagnosis of multiple myeloma, to guide the therapy, to provide prognostic information, and to monitor treatment efficacy.[4] Immunophenotyping has become an invaluable tool in the management of hematological malignancies and is increasingly finding a role in the diagnosis and monitoring of plasma cell disorders.[5]

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Immunophenotyping by flow cytometry is a sensitive method that is used for the diagnosis and clinical monitoring of the disease. Flow cytometry in multiple myeloma is beneficial in detecting malignant plasma cells and prognostic markers and monitoring the development and differentiation of myeloma cells.[6]

Flow cytometry has many advantages:

- distinguishing among normal, reactive, and malignant plasma cells;[7-11]
- evaluating the risk of progression from monoclonal gammopathy of unknown significance (MGUS) to multiple myeloma;[12-14]
- detecting prognostic markers;[10, 15-17]
- evaluating minimal residual disease (MRD);[7, 18, 19]
- identifying new targets for myeloma therapy.[10, 17, 20]

Multiple myeloma is not uncommon globally as well as in Myanmar. Being limited availability of laboratory diagnostic facility, there had been no studies using flow cytometry immunophenotyping for diagnosis of multiple myeloma in Myanmar. The study was aimed to provide an accurate diagnosis of multiple myeloma by application of flow cytometry immunophenotyping.

MATERIALS AND METHODS

Study population

A cross-sectional, descriptive study was conducted at Department of Clinical Haematology, Yangon General Hospital from September 2015 to August 2016. A total of 40 clinically suspected cases of multiple myeloma were included in the study. After getting informed consent, relevant clinical history, physical examination and laboratory results were recorded in case report form. Then, 2 ml of bone marrow aspirate with EDTA tube and 2 ml of venous blood with plain tube were collected and sent to Blood Research Division, Department of Medical Research within 2 hours.

Flow cytometry immunophenotyping

Plot configuration and optimization including isotype control, fluorescent (colour) control, calibration beads and compensation were firstly performed. After getting the standard optimized setting of machine, the patient's samples were analyzed throughout the process. Accurate and consistent test results were checked and standardized over time regardless of variables.

Immunophenotypic evaluation was performed using multicolour flow cytometry CyFlow® Cube 8 (Sysmex Partec). Multiparametric flow cytometry immunophenotyping was performed using monoclonal antibodies against CD56, CD19, CD138, CD38 and CD45 conjugated with phycoerythrin (PE), allophycocyanin (APC), fluorescein isothiocyanate (FITC), and phycoerythrin (PE-Dy).

Data were analyzed by CyView™ software. The plasma cells were initially gated using CD138 and side scatter, following which, CD138+ gated cells were analyzed for CD56, CD19 and CD45. In cases of CD138- specimens, the bone marrow aspirate was restrained with a CD56/CD19/CD38/CD45 panel. In this case, plasma cells were gated using CD38 and side scatter and CD38+ cells were analyzed for CD56, CD19 and CD45.

Serum protein electrophoresis

Visual detection of a monoclonal band following serum protein electrophoresis was used to confirm the presence of monoclonal protein. The concentration of monoclonal protein was quantified by using a densitometer.

Statistical analysis

Data entry was performed and checked for double entry, incorrectness and incompleteness to validate the data. Data were analyzed by using SPSS 16.0. Simple descriptive analysis for each variable was done.

Ethical consideration

This study was approved by Ethics Review Committee, Department of Medical Research.

RESULTS

A total of 40 clinically suspected cases of multiple myeloma were included for the study. Among the 40 cases, 25 cases (62.5%) were diagnosed as multiple myeloma by the positive expression of CD138 or CD38, negative CD19 expression, weak or negative CD45 expression and positive or negative CD56 expression (Fig. 1-3). Fifteen cases (37.5%) had no evidence of multiple myeloma by

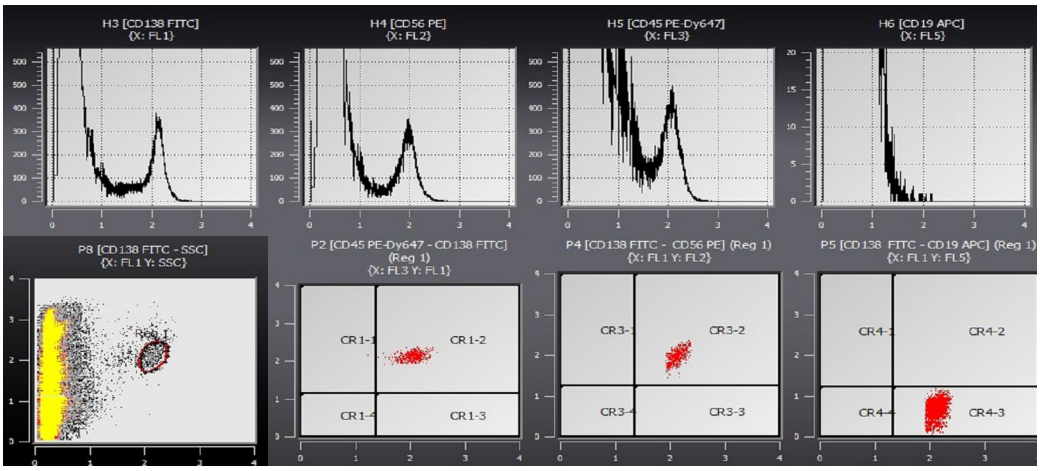

Fig.1. Phenotype of neoplastic plasma cells showing expression of CD56+ CD19- CD138+ CD45+

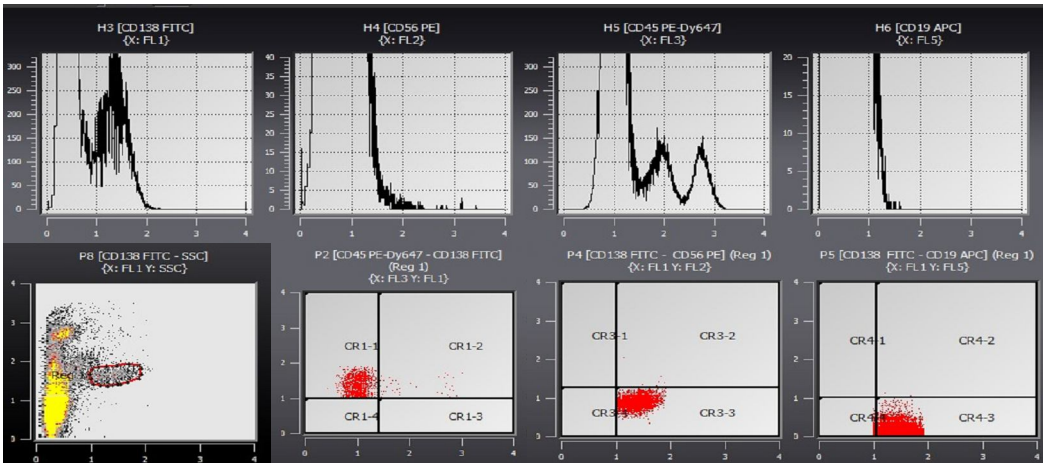

Fig. 2. Phenotype of neoplastic plasma cells showing expression of CD56- CD19- CD138+ CD45-

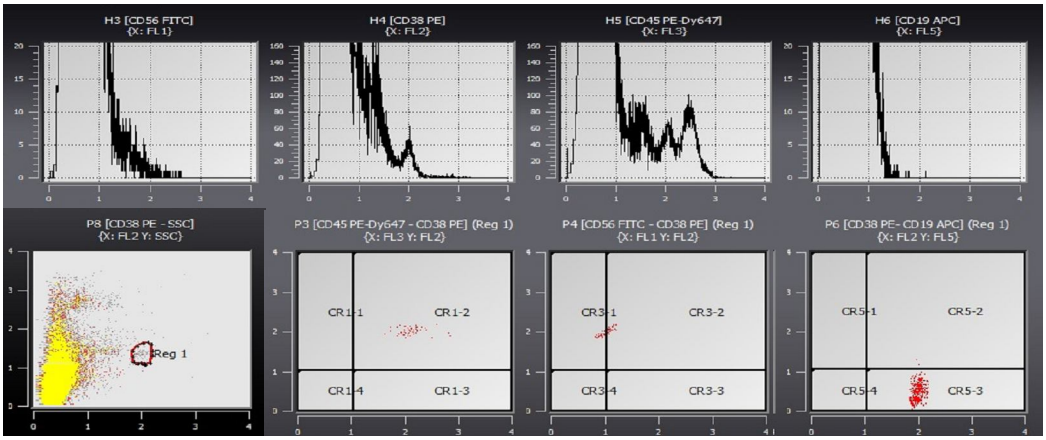

Fig. 3. Phenotype of neoplastic plasma cells showing expression of CD56+ CD19- CD38+ CD45+

flow cytometry. Age of the patients ranged from 49 years to 89 years. The male to female ratio was 1.1:1. In 23 out of 25 cases (92%), plasma cells could be sufficiently identified through initial CD138 gating. The positive expression rates of CD56, CD19, CD138 and CD45 in neoplastic plasma cells were 84% (21/25), 0% (0/25), 92% (23/25) and 32% (8/25), respectively (Table 1). Two of 25 cases (8%) were negative for CD138 expression following initial CD138 gating and were subsequently restained and gated using CD 38.

Table 1. Antigenic expression rates in multiple myeloma cases

Antigen	Expression	Multiple myeloma patients (%)
CD138	Positive	23(92)
	Negative	2(8)
CD56	Positive	21(84)
	Negative	4(16)
CD19	Positive	0(0)
	Negative	25(100)
CD45	Positive	8(32)
	Negative	17(68)

Serum protein electrophoresis and densitometer reading were performed in all cases for detection of monoclonal band and monoclonal protein concentration. Monoclonal band was detected visually and estimation of monoclonal protein was done by densitometer. Among 40 cases, 25 cases (62.5%) had monoclonal gammopathy. Among them, 20 cases (80%) had monoclonal band in the gamma (γ) region and 5 cases (20%) had in the beta (β) globulin region. The mean concentration of monoclonal protein was 4.50 g/dl, with a range of 2.28 to 7.95 g/dl. The remaining 15 cases (37.5%) were not detected monoclonal band.

DISCUSSION

Immunophenotyping has become an invaluable tool in the management of hematological malignancies and is increasingly finding a role in the diagnosis and monitoring of plasma cell disorders. The major advantage of flow cytometry when compared to other methods is the possibility to discriminate between normal polyclonal and abnormal clonal plasma cells. In addition to establishing the diagnosis of plasma cell disorders, several studies have reported an association between the phenotype of neoplastic plasma cells and prognosis.

This study demonstrated the four-color flow cytometry using monoclonal antibodies against CD56, CD19, CD138 (CD38) and CD45 to detect the neoplastic plasma cells in patients with multiple myeloma.

The European Myeloma Network has recommended using CD38, CD138 and CD45 together with CD19 and CD56 to identify multiple myeloma cells.[7] Minimum of 4 markers is recommended for basic plasma cell analysis so that expression of CD56, CD19, CD138 (CD38) and CD45 should be analyzed in every monoclonal gammopathy case to identify CD138+ CD38+ plasma cells and to discriminate normal or reactive plasma cells (CD19+ CD56 - CD45+) and abnormal plasma cells (CD19 - CD56 + or - CD45 - or +).[7, 21]

In all multiple myeloma cases, a single antigen cannot be used to distinguish neoplastic plasma cells. Although CD138 is expressed at high levels on plasma cells, [22] this marker cannot be used to discriminate neoplastic myeloma cells from reactive plasma cells. In our study, CD138 was used for the initial identification of plasma cells because plasma cells are the only cells in the bone marrow that express CD138. Subsequent selection using CD38 was analyzed only for samples with dim to negative CD138 expression. In this study, 23 of 25 cases (92%) showed CD138+ neoplastic plasma cells, with only 2 cases (2/25,8%) with CD138- neoplastic plasma cells. However, this later group showed CD38+ neoplastic plasma cells and therefore, in these cases, CD38 was used for selection. No difference was noted in the immunophenotypic profile between CD38+ cells and cells gated using the CD138 marker.

CD56 is expressed mainly on neoplastic plasma cells although not in all cases. CD56 expression was reported in 70 to 80% of multiple myeloma patients. [23] This study showed that CD56 expression was 84%. Lack of CD56 expression in myeloma has been associated with a worse prognosis.[24]

Similarly, both CD45- and CD45+ neoplastic plasma cell populations have been described. Because of the varied expression patterns of CD45 on neoplastic plasma cells, there was some limitation in identifying them using CD45 alone, unlike the case with CD56 and CD19. However, the presence of CD45- neoplastic plasma cells has been associated with poor clinical outcome.[25] In this study, CD45 expression was found in 32% of multiple myeloma patients.

Reactive plasma cells express CD19; however, neoplastic plasma cells show no or only a dim CD19 expression.[12, 26] CD19 represents the most valuable antigen to identify neoplastic plasma cells in patients with multiple myeloma. These findings suggest that CD19 expression was negative in all patients.

Conclusion

This study revealed the diagnostic role of flow cytometry immunophenotyping in patients with multiple myeloma. As an adjunct to morphologic evaluation of marrow aspirate smear, histology, immuno-histochemistry of marrow biopsy and protein electrophoretic analyses, flow cytometry immunophenotyping is useful tool for the diagnosis of multiple myeloma and it should be included as a routine assay in monoclonal gammopathy patients.

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