

## Immunophenotypic Expression Patterns and Their Clinical Correlates in Newly Diagnosed Multiple Myeloma Patients

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### Abstract

**Background:** Immunophenotypic analysis using flow cytometry is essential for evaluating newly diagnosed multiple myeloma (MM). It helps detect aberrant plasma cell populations, determine clonality, and assess the bone marrow microenvironment, thereby aiding in accurate diagnosis, prognostication, and treatment planning. This study was conducted to determine the immunophenotypic profile in newly diagnosed Multiple Myeloma by flow cytometry in a tertiary care hospital.

**Methods:** This cross-sectional analytic study was conducted in the Department of Haematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, over a period of 12 months from July 2023 to June 2024. A total of 39 newly diagnosed cases of multiple myeloma were included after getting informed written consent. Data were collected in separated case-record form and analyzed by SPSS-26.

**Results:** The mean age of the respondents was 59.13±10.77 years with male predominance (66.7%). Most patients were classified as ISS stage III (53.8%), followed by stage II (33.3%) and stage I (12.8%). Immunophenotypic analysis showed that 94.9% of patients expressed CD56, and 92.3% expressed CD38. Other markers included CD117 (35.9%), CD138 (20.5%), CD28 (12.8%), CD45 (5.1%), and CD19 (2.6%). Kappa light chain expression was observed in 81.6% of patients, while 18.4% expressed Lambda. However, no significant association was found between immunophenotypic marker expression and ISS stage of multiple myeloma.

**Conclusion:** Immunophenotypic analysis demonstrated a high prevalence of CD56 and CD38 expression, along with varying levels of other markers such as CD117, CD138, and CD28.

**Keywords:** Multiple myeloma, Immunophenotyping, Flow cytometry, Plasma cells, CD markers, Aberrant expression, ISS stage.

### 1. INTRODUCTION

Multiple myeloma (MM) is one of the most prevalent hematologic malignancies and remains incurable for most patients, with survival durations ranging from a few months to longer than 10 years. It is characterized by the clonal expansion of plasma cells in the bone marrow. Flow cytometry enables the identification and characterization of these abnormal plasma cells

based on their immunophenotypic profile. This technique utilizes fluorescence-labeled antibodies specific to different cell surface markers, allowing for the simultaneous analysis of multiple parameters in individual cells [1].

According to European Myeloma Network recommendation for the diagnosis of MM by flow cytometry, antigens such as CD138, CD38, CD33, CD19, CD56, CD45, CD117, CD20, and

CD28 must be used in the diagnostic panel [1]. In an Indian study shows frequency of immunophenotyping markers, like CD81 (93.6%), CD27 (89.3%), CD56 (79.1%), CD117 (31.9%) and CD45 (14.6%) [2]. Another study found that mean abnormal plasma cell (APC) percent in MM cases was 96.5% and sensitivity for APC detection was highest for CD19 (95.2%) followed by CD56 (90.4%) and CD81 (83.7%) [2]. In addition, antigens, such as CD45, CD56, CD117, and CD28, have been identified as prognostic markers for MM [3, 4]. But such studies are rare in Bangladesh.

In case of multiple myeloma immunophenotyping helps in the detection of aberrant plasma cell populations and the assessment of their clonality. The typical immunophenotypic profile of normal plasma cells include the expression of CD38 and CD138, while CD45 is usually negative. Aberrant plasma cells often show decreased expression of CD45 and increased expression of CD38 and CD138. Additionally, the evaluation of other markers such as CD56, CD19, and CD20 helps differentiate between normal and abnormal plasma cells [5, 6, 7].

MM is characterized by the expansion of a clonal population of plasma cells. Immunophenotyping is important for the assessment of plasma cell clonality. In a normal immune system, B-cell development and maturation lead to the production of polyclonal plasma cells. This clonality can be determined by evaluating the expression of immunoglobulin light chains (kappa and lambda) on plasma cells. A restricted expression of either kappa or lambda light chains indicates clonality and supports the diagnosis of MM [8, 9].

In addition to the detection and clonality assessment of plasma cells, Immunophenotyping can provide valuable information about other cell populations in the bone marrow microenvironment. It allows for the analysis of immune cell subsets such as T cells, B cells, natural killer (NK) cells, and dendritic cells. Alterations in the proportions and phenotypes of these immune cells can have prognostic implications and may influence disease progression and response to treatment [10].

Immunophenotypic analysis can be combined with other techniques to enhance the diagnostic accuracy and prognostic value in MM, which involves the evaluation of multiple markers simultaneously, improves the identification of abnormal plasma cell populations, the incorporation of multiparameter flow cytometry immunophenotyping into the routine diagnostic

evaluation of MM patients can help to identify patients at a high risk of progression. The aim of this study is to observe the immunophenotypic pattern in newly diagnosed multiple myeloma.

## 2. OBJECTIVE

The objective of this study was to determine the immunophenotypic profile in newly diagnosed Multiple Myeloma by flow cytometry in a tertiary care hospital.

## 3. METHODOLOGY & MATERIALS

This cross-sectional analytic study was conducted in the Department of Haematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, over a period of 12 months from July 2023 to June 2024. A total of 39 newly diagnosed cases of multiple myeloma were included according to the International Myeloma Working Group (IMWG) 2022 criteria. Patients with previously treated multiple myeloma, concomitant malignancies, monoclonal gammopathy of undetermined significance, smoldering multiple myeloma, and plasma cell leukemia were excluded. The sample was collected consecutively using purposive sampling techniques. Informed written consent was obtained from each participant after explaining the study's aim, purpose, and procedure. Data were collected using a semi-structured questionnaire and checklist, which included socio-demographic profiles, clinical features, and comorbidities such as hypertension, diabetes mellitus, heart disease, liver disease, renal disorder, and respiratory disease. Blood investigations including complete blood count, serum calcium, creatinine, albumin, globulin, lactate dehydrogenase (LDH), and  $\beta$ 2-microglobulin were performed, and staging was done according to the International Staging System (ISS). Complete blood count was done using the SYSMEX XN-2000 hematology analyzer. Bone marrow aspiration was performed from the posterior superior iliac crest, and the first 2 ml of the sample was collected in an EDTA tube for flow cytometric immunophenotyping. The immunophenotyping of plasma cells was carried out using multiparameter flow cytometry (BD FACSLyric with BD FACSuite software). The diagnostic panel included markers CD19, CD20, CD28, CD38, CD44, CD45, CD52, CD56, CD117, CD138, kappa, and lambda. Bone marrow smears were stained with Leishman stain and examined microscopically. Quality assurance measures were maintained for all instruments. Data were checked, verified for consistency, and

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analyzed using SPSS version 26. Continuous variables were expressed as mean and standard deviation, while categorical data were expressed as frequency and percentage. Associations between categorical variables were assessed using the chi-square test, with a p-value of <0.05 considered statistically significant. Ethical approval was obtained from the Institutional Review Board (IRB) of BSMMU, and confidentiality of all participants was strictly maintained throughout the study.

### 4. RESULTS

In this study of 39 newly diagnosed multiple myeloma patients, the mean hemoglobin was

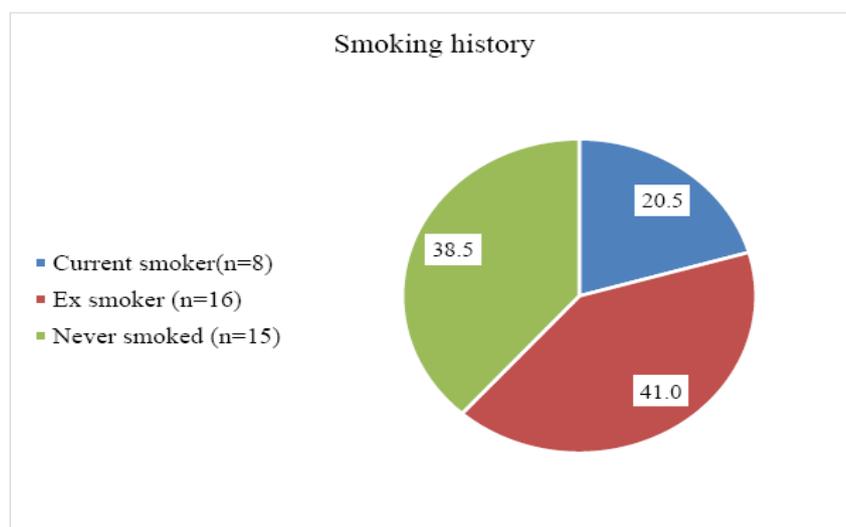
8.77±1.99 g/dl, ESR 109.87±31.75 mm, LDH 328.23±193.68 U/L, and serum creatinine 2.89±2.27 mg/dl. Free light chain ratio, IgG, IgA, IgM, and  $\beta$ 2-microglobulin were 1.5±0.87, 41.88±21.42, 0.32±0.18, 0.25±0.22, and 6.76±3.66, respectively. Immunophenotypic analysis showed high expression of CD56 (94.9%) and CD38 (92.3%), with lower frequencies of CD117, CD138, CD28, CD45, and CD19. Kappa light chain predominated (81.6%). No significant association was found between immunophenotype expression and ISS stage, highlighting the diagnostic value of flow cytometry while suggesting further prognostic studies.

**Table 1.** Sociodemographic condition of the respondents (n=39)

Variable	Mean±SD	Range
Mean age (year)	59.13±10.77	40-78
<b>Gender</b>	Frequency (n)	Percentage (%)
Male	26	66.7
Female	13	33.3
<b>Area of residence</b>		
Urban	8	20.5
Rural	31	79.5
<b>Occupation</b>		
Farmer	5	12.8
Housewife	13	33.3
Businessman	8	20.5
Service holder	7	17.9
Retired	5	12.8
Others	1	2.6
<b>Monthly family income</b>		
10000 to less than 20000 tk	14	35.9
20000 to less than 40000 tk	18	46.2
More than 40000 tk	7	17.9

Table 1 shows the mean age of the respondents was 59.13±10.77 years with the range of 40-78 years. Also majority of the respondents were male (66.7%) and resided from rural area (79.5%).

According to occupational status, 33.3% were housewife and 20.5% were businessman. However, most of the respondents had monthly family income of 20000 to 40000 tk per month (46.2%).



**Figure 1.** Distribution of smoking history of the respondents (n=39)

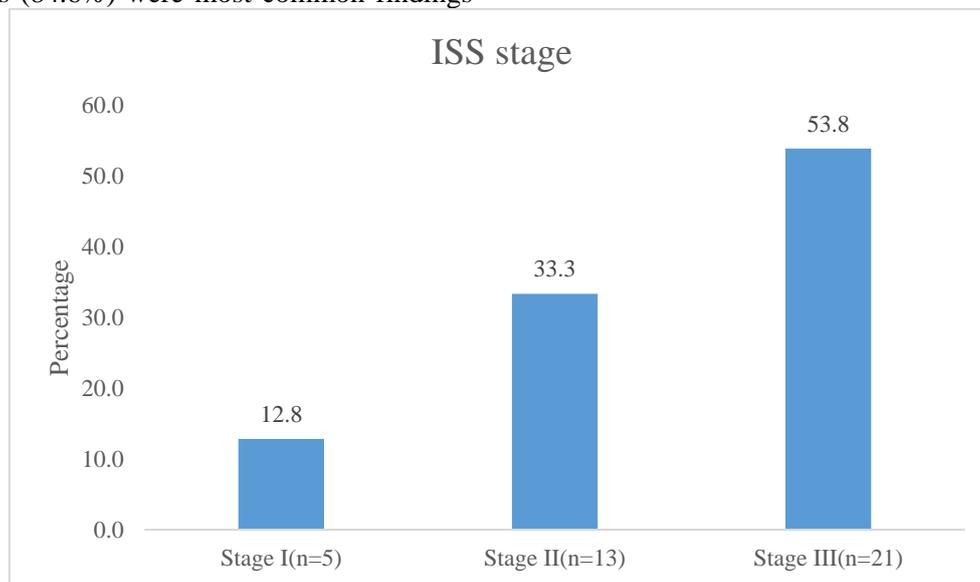
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Only 8 respondents out of 39 were current smokers (20.5%). While majority of the respondents were ex-smoker (41%) and 38.5% were non smoker (Figure 1).

**Table 2.** Distribution of clinical feature of the respondents (n=39)

Variables	Frequency (n)	Percentage (%)
Bone pain	37	94.9
Anaemia	35	89.7
Weakness	33	84.6
Fatigue	23	59
Weight loss	17	43.6
Frequent infection	9	23.1

Multiple responses were considered among respondents. Also fatigue (59%), weight loss (43.6%) and frequent infection were seen among respondents in this study (Table 2). Bone pain (94.9%), anemia (89.7%) and weakness (84.6%) were most common findings



**Figure 2.** ISS stage distribution of the respondents (n=39)

More than half of the respondents were ISS stage III (53.8%) followed by stage II (33.3%) and stage I (12.8%) (Figure 2).

**Table 3.** Laboratory findings of the respondents (n=39)

Variables	Mean±SD
Hb (g/dl)	8.77±1.99
Total RBC count (X10 <sup>12</sup> mg/dl)	3.1±0.78
Total WBC count (X10 <sup>9</sup> /cumm)	7.78±2.53
Neutrophil count (X10 <sup>9</sup> /cumm)	4.6±1.94
Lymphocyte count (X10 <sup>9</sup> /cumm)	2.52±1.19
Platelet count (X10 <sup>9</sup> /cumm)	230.03±101.92
ESR	109.87±31.75
LDH (U/L)	328.23±193.68
S. Creatinine (mg/dl)	2.89±2.27
S. Calcium (mg/dl)	8.66±3.4
S. Albumin (g/L)	28.76±12.84
Free light chain ratio	1.5±0.87
Clonal plasma cell percentage	14.42±12.49
IgG	41.88±21.42
IgA	0.32±0.18
IgM	0.25±0.22
β2 microglobulin	6.76±3.66
Plasma cell dyscrasia (%)	57.82±22.67

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The mean hemoglobin level was  $8.77 \pm 1.99$  g/dl and ESR level was  $109.87 \pm 31.75$  mm in 1st hour. Also LDH and serum creatinine were  $328.23 \pm 193.68$  U/L and  $2.89 \pm 2.27$  mg/dl

respectively. Free light chain ratio, IgG, IgA, IgM and  $\beta 2$  microglobulin were  $1.5 \pm 0.87$ ,  $41.88 \pm 21.42$ ,  $0.32 \pm 0.18$ ,  $0.25 \pm 0.22$  and  $6.76 \pm 3.66$  accordingly (Table 3).

**Table 4.** Common immunophenotype expression pattern on plasma cells in newly diagnosed multiple myeloma patients (n=39)

Variables	Frequency (n)	Percentage (%)
CD19	1	2.6
CD28	5	12.8
CD38	36	92.3
CD45	2	5.1
CD56	37	94.9
CD117	14	35.9
CD138	8	20.5
Kappa	31	79.5
Lambda	7	17.9

According to common immunophenotype, 37 (94.9%) had CD56 marker and 36(92.3%) had CD38 marker. Also, 14 respondents had CD117, 8 respondents had CD138, 5 respondents had

CD28, 2 respondents had CD45 and 1 respondent had CD19. Also, 31 respondents had Kappa expression and 7 respondents had Lambda expression (Table 4).

**Table 5.** Association between immunophenotype expression with ISS stage (n=39)

Variables	ISS stages			p value
	Stage I n (%)	Stage II n (%)	Stage III n (%)	
CD19	0	0	1(4.8)	0.644
CD28	2(40)	2(15.4)	1(4.8)	0.100
CD38	5(100)	13(100)	18(85.7)	0.248
CD45	1(20)	0	1(4.8)	0.225
CD56	5(100)	12(92.3)	20(95.2)	0.798
CD117	3(60)	7(53.8)	4(19)	0.059
CD138	2(40)	2(15.4)	4(19)	0.496
Kappa	3(60)	11(84.6)	17(81)	0.496
Lambda	2(40)	2(15.4)	3(14.3)	0.387

Chi square test was done. Values were expressed in frequency with percentage in parenthesis over column. However, there was no significant association found between immunophenotype expressions with ISS stage of multiple myeloma (Table 5).

### 5. DISCUSSION

Multiple myeloma (MM) is a complex and heterogeneous haematologic malignancy characterized by the clonal proliferation of malignant plasma cells [11]. The clinical and biological features of MM can vary significantly, as evidenced by the demographic, clinical, and immunophenotypic characteristics presented in this study from a tertiary care hospital. The mean age of the respondents in this study was 59.13 years, which is consistent with the typical age of MM onset, generally diagnosed in the sixth

decade of life. However, Khallaf et al., also found the median age of MM patients was 52 years (range 32-75) in his study which similar to this study findings [12]. This aligns with global data indicating that MM predominantly affects older adults, with a median age at diagnosis between 65 and 70 years [13, 14]. However, the range of 40-78 years suggests the presence of MM in a somewhat younger cohort within this population, which could be attributed to genetic, environmental, or socio-economic factors specific to the region.

The male predominance (66.7%) observed in this study is also consistent with global trends, where MM tends to have a higher incidence in males than females. The higher proportion of respondents from rural areas (79.5%) highlights potential disparities in healthcare access, awareness, and diagnostic facilities between urban and rural populations. Occupational status

data revealed that a significant portion of respondents were housewives (33.3%) and businessmen (20.5%), reflecting the socio-economic status of the population. The majority had a monthly family income of 20,000 to 40,000 taka (46.2%), indicating that most patients belong to lower-middle-income households. Montes-Gaisán et al., median age of multiple myeloma patients was 73 (9.73) years, 103 (49.8%) patients were male and 104 (50.2%) female 49 (23.9%) and 156 (76.1%) lived in rural and urban areas [13]. The smoking history among respondents showed that only 20.5% were current smokers, with a larger proportion being ex-smokers (41%). While smoking is not directly associated with MM, its role in general health deterioration and potential interactions with other risk factors warrants further investigation. Non-smokers made up 38.5% of the cohort, suggesting that smoking is not a predominant risk factor in this population.

The clinical presentation of MM in this cohort was typical, with bone pain (94.9%), anemia (89.7%), and weakness (84.6%) being the most common symptoms. These findings are consistent with the literature, where bone pain is often the presenting symptom due to osteolytic lesions caused by plasma cell proliferation [15]. Anemia, resulting from bone marrow infiltration and renal impairment, is also a common feature of MM and contributes to the overall fatigue and weakness reported by patients. Interestingly, the prevalence of hypertension (43.6%) and diabetes mellitus (41%) was relatively high among the respondents, yet no cases of heart, renal, or liver disease were reported. Also Sverrisdóttir et al., found most common comorbidities were hypertension (20.4%) [16]. As the prevalence of multimorbidity increases with age, patients with MM are more likely to be affected by comorbid chronic disease which might also affect the quality of life of the patients [14].

However, this study found ISS stage for multiple myeloma was most common among stage III (53.8%) followed by stage II (33.3%) and stage I (12.8%). This was also aligned with Meddour et al., where majority were in stage III 50/112 patients and in stage I 49/112 patients [17].

The mean hemoglobin level of 8.77 g/dl indicates significant anemia, which is a common finding in MM due to the suppression of erythropoiesis by malignant plasma cells and the effects of chronic disease. The elevated ESR (109.87 mm in 1st hour) is also typical of MM, reflecting the chronic inflammatory state and high levels of circulating monoclonal proteins.

LDH and serum creatinine levels (328.23 U/L and 2.89 mg/dl, respectively) were elevated, indicating some degree of tissue damage and possible early renal impairment. LDH is a marker of cell turnover and has been associated with poor prognosis in MM, particularly in cases with aggressive disease. The creatinine level suggests the need for careful monitoring of renal function, even though no overt renal disease was reported.

The free light chain ratio, IgG, IgA, IgM, and  $\beta$ 2 microglobulin levels provided further insight into disease burden and prognosis.  $\beta$ 2 microglobulin, in particular, is a well-established prognostic marker in MM, with higher levels associated with advanced disease and poorer outcomes. The mean level of 6.76 mg/L in this cohort is indicative of a high disease burden, correlating with the high percentage of patients in ISS stage III.

The immunophenotypic analysis revealed that CD56 and CD38 were the most commonly expressed markers (94.9% and 92.3%, respectively). These markers are typically associated with normal and malignant plasma cells, with CD56 often linked to more aggressive disease and poorer outcomes. Rath et al., showed CD19 was the most sensitive (100%) and CD81 was the most specific marker (100%) for differentiating abnormal plasma cells (APCs) among multiple myeloma patients [2]. Also in Mengich et al., Of the 83 selected cases, expression of Cyclin D1, CD56, CD117 and Ki-67 was identified in 28.9, 34.9, 7.2, and 50.6%, respectively [18]. The expression of CD117, CD138, CD28, CD45, and CD19 in smaller subsets of patients highlights the heterogeneity of MM and its varied immunophenotypic profiles. The absence of a significant association between these markers and ISS stage suggests that while these markers are helpful for diagnosis, they may not be directly predictive of disease stage or progression. However, absence of CD117 expression was noted to be associated with adverse risk parameters including an IgA isotype or light chain disease, International Staging System (ISS) stage III disease, abnormal baseline serum free light chains (sFLC) and a high plasma cell burden [18].

## 6. LIMITATIONS OF THE STUDY

This study was limited by its small sample size, single-center design, and the use of purposive sampling without randomization, which may affect the generalizability of the findings. Future research should encourage the routine use of flow cytometry for immunophenotypic profiling in

newly diagnosed multiple myeloma to improve diagnostic accuracy and guide personalized treatment. Expanding marker panels and conducting large-scale, multicenter studies are recommended to explore additional immunophenotypic markers and further evaluate their prognostic significance in relation to disease severity, treatment response, and survival outcomes.

### 7. CONCLUSION

Immunophenotypic analysis demonstrated a high prevalence of CD56 and CD38 expression, along with varying levels of other markers such as CD117, CD138, and CD28. The majority of patients exhibited Kappa light chain expression, while a smaller proportion had Lambda expression. Despite the distinct immunophenotypic profiles, no significant association was observed between marker expression and the ISS stage of multiple myeloma. These findings emphasize the role of flow cytometry in characterizing plasma cell populations but suggest further research is needed to understand its prognostic implications.

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### CONFLICTS OF INTEREST

There are no conflicts of interest.

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