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Multiparameter Flow Cytometry Immunophenotyping for the Identification of Smoldering Multiple Myeloma: A Case Report

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Author's contribution

The sole author designed, analysed, Interpreted and prepared the manuscript.

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Case Report

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ABSTRACT

Smoldering multiple myeloma (SMM) is a clonal plasma cell (PC) condition that falls between monoclonal gammopathy of unknown significance (MGUS) and multiple myeloma (MM). Detecting SMM patients allows for early therapy, which slows the disease's course and reduces morbidity**.** This report illustrates the case of a 68-year-old male who presented with suspected plasma cell dyscrasia. Multiparameter flow cytometry (MFC) immunophenotypic characteristics of bone marrow, absence of cytogenetic abnormalities (low risk) with no evidence of CRAB features (hypercalcemia, renal insufficiency, anemia, bone lesion), confirmed the diagnosis of smoldering multiple myeloma as evidenced by increased plasma cells (both mature and immature) comprising 70% of NEC of bone marrow revealed by bone marrow study, monoclonal [("M") spike band value of 5.71gm/dl] seen in Gamma region by serum protein electrophoresis and monoclonal gammopathy with IgG band and Kappa band by immunofixation. MFC's involvement in analysing neoplastic PCs is becoming increasingly important in precisely identifying and quantifying BMPCs with malignant potential from normal PCs, assisting in the diagnosis and classification of Plasma cell dyscrasias.

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1. INTRODUCTION

Smoldering multiple myeloma (SMM) is a clonal plasma cell (PC) disorder [1] defined by the presence of >_3g/dL serum monoclonal (M) protein and/or 10% to 60% clonal bone marrow PCs (BMPCs) with no evidence of end-organ damage (i.e., CRAB criteria include hypercalcemia, renal insufficiency, anaemia, bone lesion) or other " (MDE) [2].

SMM in 1980 was initially described as an intermediate stage between monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM), with a higher disease burden [3]. SMM is less common than MGUS, representing an estimated 13.7% of patients with MM, with 4,100 new patients per year [4]. The rate of progression to active MM is 10% per year for the first five years, declines to 3% per year for the next five years, and is then 1% per year for the following ten years. The cumulative probability of progression from SMM to MM is 73% at 15 years [1].

Recent trials in SMM showed that early therapy could be potentially beneficial to patients. So, it is essential to diagnose accurately and risk-stratify patients with SMM by routinely incorporating modern imaging (MRI, PET CT) and laboratory techniques, including serum-free light chain assay, serum protein electrophoresis with immunofixation and quantitation of immunoglobulins, Flow cytometry immunophenotyping, cytogenetic studies [5]. The role of Immunophenotyping by multiparameter flow cytometry (MFC) to evaluate neoplastic plasma cells becomes invaluable in diagnosing and classifying these disorders, assessing their prognosis, monitoring disease burden, and responding to ongoing therapy [6]. Flow cytometry analyzes the expression of several markers in bone marrow plasma cells, including CD45, CD19, CD138, CD38, CD56, cytoplasmic kappa light chain, and cytoplasmic lambda light chain. In the bone marrow, only plasma cells express a high level of CD138, which is used to identify and isolate these populations for initial identification. CD38 is a non-specific marker expressed on the surface of both hematopoietic and nonhematopoietic cells. Neoplastic plasma cells displayed aberrant/overexpression of CD56 but no or dim CD19 expression and variable expression of CD45 (CD45-/ CD45+). CD45 negativity is associated with poor clinical outcomes. So, Immunophenotyping with MFC plays a significant role in SMM to accurately distinguish and quantitate neoplastic BMPCs as well as helps to determine prognosis [7].

MFC is included in the initial diagnostic workup along with the patient's clinical history, other laboratory results, and a bone marrow study because of its ability to provide relatively fast and conclusive results, which aid in distinguishing malignant from reactive conditions as well as classifying different monoclonal gammopathies and other lymphoproliferative disorders. As a result, MFC has become increasingly important in plasma cell dyscrasias [8].

This case report presents the potential utility of multiparameter flow cytometry (MFC) immunophenotyping in diagnosing SMM by correlating with clinical features, serum calcium, peripheral blood smear findings, bone marrow study, urine analysis for Bence Jones Protein and bone lesions. MFC immunophenotyping also accurately distinguishes and quantitates BMPCs that have malignant potential from normal PCs.

2. CASE REPORT

A 68-year -old Asian male was admitted to Bangabandhu Sheikh Mujib Medical University (BSMMU) Hospital, Bangladesh, under hematologist in August 2019 with complaints of generalized weakness, fever (temperature 99◦ F) for two weeks, cough, swelling of the leg, anemia, malena but no bone pain. The patient was conscious, able to walk, cooperative on admission, mentally sound. On physical examination, there was no organomegaly and lymphadenopathy. The remainder of the physical examination was unremarkable. Past medical history showed he had hypertension since 2004, acute myocardial infarction since 2006, diabetes mellitus type 2 since 2010, atrial fibrillation since 2014, and had an ischemic stroke in 2014. Coronary artery bypass surgery was done in 2006.

Laboratory investigations: initial laboratories after symptoms started were remarkable for unexplained normocytic/normochromic anemia (9.2 g/dL) with rouleaux formation, and white blood cells and platelets were within normal limits. He had an elevated erythrocyte

sedimentation rate (114 mm/1 h). An occult blood test was positive, which indicates that the anemia may be due to malena. Serum creatinine level was within normal range, 0.81mg/dl (ref. range: 0.60-1.40 mg/dl). A mild decrease in serum calcium level was 7.40mg/dl (ref. range: 8.50-11.0 mg/dl). Serum sodium, potassium, and chloride level were within normal limits. Total protein in serum was elevated, 12.1gm/dl (ref. range: 6.4-8.3gm/dl). Serum immunoglobulin profile showed increased level of IgG, 65.1gm/l (ref. range: 7.0-16.0 gm/l), but decreased level of IgA, <0.25gm/l (ref. range: 0.7- 4.0 gm/l) and IgM, < 0.17 gm/l (ref. range: 0.4-2.3gm/l). Bence Jones protein in urine was absent. Urinary albumin: creatinine ratio was 6.15mg/gm (Table 1).

Bone marrow study revealed an increase in plasma cells (both mature and immature), comprising 70% of NEC of bone marrow, suggestive of plasma cell dyscrasia. An Ultrasonogram of the whole abdomen revealed the bilateral renal parenchymal disease. No bony lesion was seen in MRI and x-ray of skull and pelvis. X-ray showed features of consolidation in the lung.

Additional laboratory tests were performed for further evaluation because of this patient's bone marrow study result, unexplained anaemia, and increased total serum protein; these included serum protein electrophoresis with immunofixation and quantitation of immunoglobulins, flow cytometry immunophenotyping, and cytogenetic studies. Serum protein electrophoresis revealed a normal Beta-globulin, 0.38gm/dl (ref. range: 0.30- 0.59 gm/dl) and monoclonal [("M") spike band value of 5.71gm/dl] seen in Gamma region. There was an elevated level of Gamma globulin, 6.79gm/l (ref. range: 0.71- 1.54 gm/l). Albumin/Globulin ratio was 0.51 (ref. range: 1.0-2.2). Serum immunofixation revealed monoclonal gammopathy with IgG and Kappa band (Table 1). A free Kappa light chain with a Kappa/Lambda ratio 19.00 (ref. range: 0.26-1.65) in serum-free light chain assay. Chromosomal analysis showed negativity for del(13q), del(17p), FGFR3 and IgH translocations [t(4;14), t(14;20), t(14;16)]. This patient had no cytogenetic abnormalities (low risk).

Bone marrow specimen was analyzed by 4-color flow cytometry, using cluster analysis of ungated data, for the expression of several markers including CD45, CD19, CD138, CD38, CD56, cytoplasmic kappa light chain, and cytoplasmic lambda light chain. MFC analysis revealed a distinct population of cells of which more than

Table 1. Laboratory test results upon initial presentation

Laboratory tests	Result	Reference range
White blood cells	6.41×10^3/cmm	4.0- 11.0×10^3/cmm
Hemoglobin	9.2 g/dL	13- 18g/dl
Red blood cells	3.02×10^6/cmm	4.5- 6.5×10^6/cmm
Platelet	183×10^3/cmm	150- 450×10^3/cmm
Serum creatinine	0.81mg/dl	0.60- 1.40 mg/dl
Serum calcium	7.40mg/dl	8.50- 11.0 mg/dl
Total serum protein	12.1gm/dl	6.4- 8.3gm/dl
Albumin	4.1g/dl	3.57- 5.42g/dl
Beta Globulin	0.38g/dl	0.30- 0.59g/dl
Gamma Globulin	6.79g/dl	0.71- 1.54g/dl
Albumin/Globulin ratio	0.51	1.0- 2.2
Serum immunofixation	Monoclonal Gammopathy with IgG and Kappa band	Absent
Monoclonal M-spike band in Gamma region in serum protein electrophoresis	5.71g/dl	Absent
Bence Jones protein in the urine	Absent	
Serum immunoglobulin profile:		
IgG level	65.1gm/l	7.0 - 16.0 gm/l
IgM level	< 0.17 gm/l	0.4- 2.3gm/l
IgA level	<0.25gm/l	0.7- 4.0 gm/l
Urinary albumin: creatinine ratio	6.15mg/gm	<30.0mg/gm

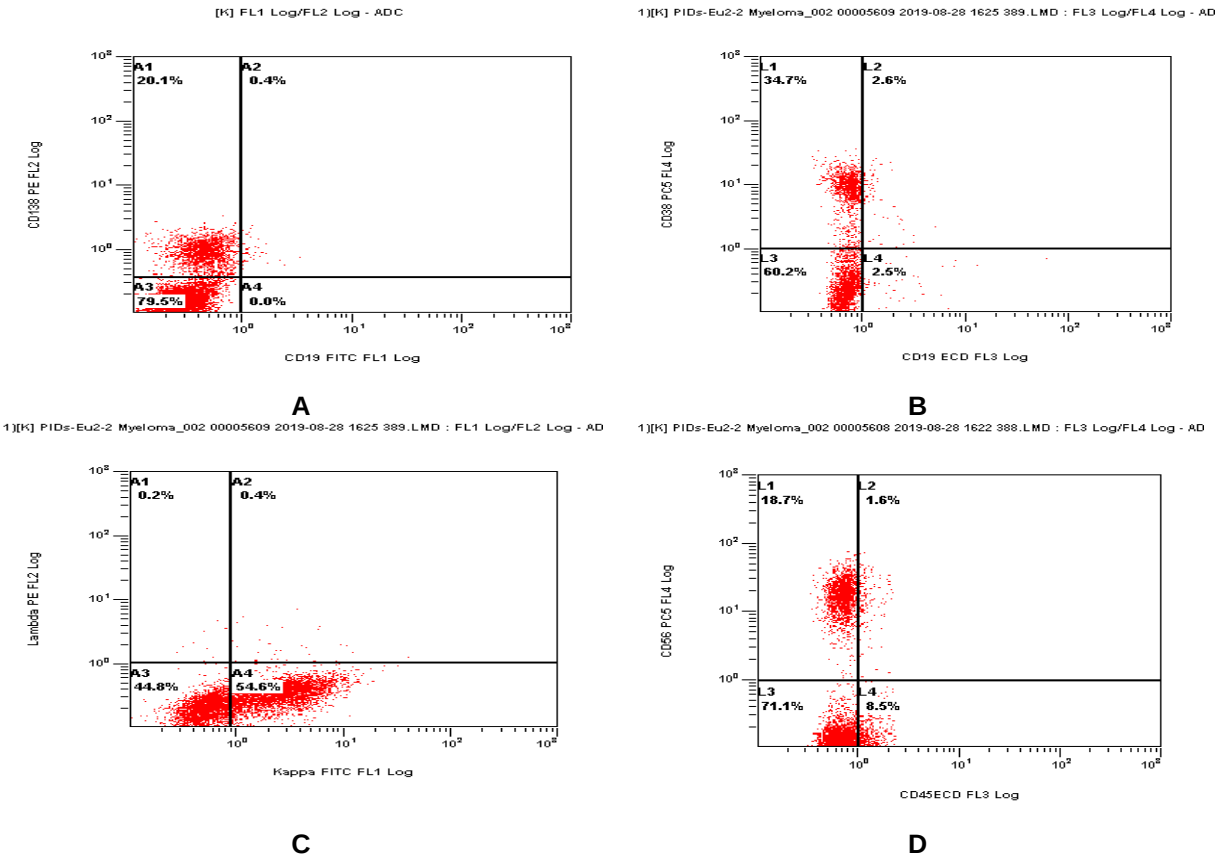

Fig. 1. Flow cytometry immunophenotyping of bone marrow: Plasma cells (PCs) are first identified by gating using CD45, CD138, and CD38. A and B, CD138 (moderate), and CD38 (bright) expression identify plasma cell population with downregulation of CD19 (dim/-). C and D, Dim to moderate cytoplasmic kappa light-chain restriction and overexpression of aberrant CD56 with CD45-/dim. Here, neoplastic plasma cells showed CD138+/CD38+/ CD56+/CD19-/CD45-/ kappa light chain restriction

20% of cells were expressing both CD138 (37.2%; moderate expression) and CD38 (20.5%; bright expression) that represent plasma cells (Fig. 1A, B). There was overexpression of aberrant CD56 (Fig. 1D) with dim to moderate cytoplasmic kappa light-chain restriction (Fig. 1C) and simultaneous downregulation of CD19 and CD45 (Fig. 1B, D). The overall immunophenotypic findings are suggestive of smoldering multiple myeloma.

The immunophenotypic characteristics of bone marrow, absence of cytogenetic abnormalities with no evidence of CRAB features (hypercalcemia, renal insufficiency, anemia, bone lesion), supports the diagnosis of smoldering multiple myeloma as evidenced by increased plasma cells (both mature and immature) comprising 70% of NEC of bone marrow revealed by bone marrow study, monoclonal [("M") spike band value of 5.71gm/dl] seen in Gamma region by serum protein electrophoresis and monoclonal gammopathy with IgG band and Kappa band by immunofixation. In this case report, MFC analysis revealed a distinct population of cells of which more than 20% cells were expressing both CD138 and CD38 that represent neoplastic plasma cells. But bone marrow study morphologically revealed more than 60% plasma cells. This study showed that multiparametric flow cytometry can accurately distinguish and quantitate neoplastic bone marrow plasma cells thereby can differentiate smoldering multiple myeloma from multiple myeloma.

The patient was treated with bortezomib and dexamethasone and had partial remission revealed by bone marrow examination & free light chain assay. Now the patient has been on maintenance therapy with bortezomib for two years.

3. DISCUSSION

The distinction between SMM, MGUS, and MM is essential for diagnosis, treatment, and prognosis. Identification of SMM patients offers an early treatment option that halts the disease's progression, thereby reducing morbidity.

Immunophenotyping with multiparametric flow cytometry plays a significant role in SMM determining prognosis and accurately distinguishing and quantitating neoplastic BMPCs [8]. Aberrant phenotype is defined by the absence of CD19 and CD45 expression, decreased expression of CD38, and overexpression of CD56 in neoplastic plasma cells[9-11].

In this case report, MFC analysis revealed a distinct population of cells expressing both CD138 (moderate) and CD38 (bright) that represent plasma cells. Here neoplastic plasma cells showed CD138+/CD38+/ CD56+/CD19-/CD45-/ kappa light chain restriction.

Variable expression of CD45 reported in bone marrow plasma cells of MM patients. In this case report, expression of CD45 was negative, representing the neoplastic plasma cell population in SMM; CD45 negativity is associated with poor clinical outcomes. In the bone marrow, plasma cells are the only cells that express high levels of CD138, used to identify and isolate these populations for initial identification as CD38 is a non-specific marker expressed on the surface of both hematopoietic nonhematopoietic cells. Neoplastic plasma cells displayed aberrant CD56 expression but no or dim CD19 expression. According to European Myeloma Network (EMN) recommendations, CD19 and CD56 markers are "essential" for diagnosing and monitoring multiple myeloma [12]. In MM, almost all (95%) plasma cells are clonal and have an aberrant immunophenotype, whereas, in MGUS, plasma cells are predominantly polyclonal and display normal immunophenotype [9-11]. A study on SMM found that 60% of patients with SMM have an aberrant immunophenotype similar to MM (95% PC aberrancy; 5% of the detected PCs are normal) [9].

Complete blood count, serum creatinine, serum calcium, skeletal survey, serum protein electrophoresis with immunofixation, serum FLC assay, bone marrow examination, and fluorescent in situ hybridization studies to detect high-risk cytogenetic abnormalities as well as plasma cell immunophenotyping by multiparametric flow cytometry to enable accurate risk stratification should all be included in the baseline studies [13].

At presentation, this case fulfilled the diagnostic criteria defined by Rajkumar et al. for SMM diagnosis based on the results of the aforesaid examinations performed [2]. The Mayo Clinic group analyzed the prognostic influence of cytogenetic abnormalities in a series of 351 patients with SMM [14].

Patients were defined as high-risk SMM with t(4;14) and/or del(17p), patients with trisomies (intermediate risk), other cytogenetic abnormalities including t(11;14) (standard risk), and no cytogenetic abnormalities (low risk). Similar findings have also been observed by another study in patients with SMM [15]. The patient of this case report had no cytogenetic abnormalities, thereby at low-risk SMM.

4. CONCLUSION

MFC's role in evaluating neoplastic PCs is becoming invaluable to accurately distinguishing and quantitating BMPCs that have malignant potential from normal PCs, thereby helping to diagnose and classify Plasma cell dyscrasias. Identification of SMM patients offers an early treatment option that halts the disease's progression, thereby reducing morbidity. In conclusion, this case emphasizes the importance of MFC analysis of bone marrow of any patient with a suspected plasma cell dyscrasia with no evidence of end-organ damage.

CONSENT

Written informed consent was obtained from the patient for the publication of the case report.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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