Case report

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

Abstract: Smoldering multiple myeloma (SMM) is an asymptomatic clonal plasma cell (PC) disorder, intermediate stage between monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM). Identification of SMM patients offer early treatment option which halts progression of the disease thereby reducing the morbidity. This report illustrates the case of a 68-year-old male 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. Role of MFC to evaluate neoplastic PCs becoming invaluable to accurately distinguishing and quantitating BMPCs that have malignant potential from normal PCs thereby helps to diagnose and classify Plasma cell dyscrasias.

Keywords: Smoldering multiple myeloma, Multiple myeloma, MFC, CD markers, Bone marrow plasma cell.

1.Introduction

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

SMM, initially described in 1980, intermediate stage between monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM), with 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 5 years, declines to 3% per year for the next 5 years, and is then 1% per year for the following 10 years. The cumulative probability of progression from SMM to MM is 73% at 15 years.[1]

Recent trials in SMM showed that early therapy can be potentially beneficial to patients. So, it is important to accurately diagnose and risk-stratify patients with SMM, by incorporating modern imaging and laboratory techniques routinely.[5] Role of Immunophenotyping by multiparameter flow cytometry (MFC) to evaluate neoplastic plasma cells becoming invaluable to diagnose and classify these disorders, to assess their prognosis, monitoring disease burden and response to ongoing therapy.[6] Along with patient's clinical history, other laboratory results and bone

marrow study, MFC is also a part of the initial diagnostic work-up due to its capacity to provide relatively fast and conclusive results, thus helps to distinguish malignant from reactive conditions as well as classify different monoclonal gammopathies and other lymphoproliferative disorders. So, the importance of MFC has progressively increased in plasma cell dyscrasias.[7]

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

2.Case Report

A 68-year -old male was admitted in Bangabandhu Sheikh Mujib Medical University (BSMMU) Hospital, Bangladesh, under hematologist in August 2019 with complaints of generalized weakness, fever, cough, swelling of leg, anemia, malena but no bone pain. On physical examination there was no organomegaly and lymphadenopathy. Remainder of 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 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 elevated erythrocyte sedimentation rate (114 mm/1 h). Serum creatinine level was within normal range, 0.81mg/dl (ref. range: 0.60-1.40 mg/dl). There was mild decrease in serum calcium level, 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 increase in plasma cells (both mature and immature), comprising 70% of NEC of bone marrow, suggestive of plasma cell dyscrasia. Ultrasonogram of whole abdomen revealed bilateral renal parenchymal disease. No bony lesion was seen in x-ray of skull and pelvis. X-ray chest showed features of consolidation in lung.

Given the combination of this patient's bone marrow study result, unexplained anemia and increased total serum protein, additional laboratory tests were performed for further evaluation; these included serum protein electrophoresis with immunofixation and quantitation of immunoglobulins, Flow cytometry immunophenotyping, 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 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). There was free Kappa light chain with 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 20% cells were expressing both CD138 (37.2%; moderate expression) and CD38 (20.5%; bright expression) that represent plasma cells (Figure, 1A, B). There was overexpression of aberrant CD56 (Figure, 1D) with dim to moderate cytoplasmic kappa light-chain restriction (Figure,1C) and simultaneous downregulation of CD19 and CD45 (Figure,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.

The patient was treated with bortezomib and dexamethasone and had partial remission revealed by bone marrow examination & free light chain assay. Now the patient on maintenance therapy with bortezomib for 2 years.

3.Discussion

The distinction among SMM, MGUS and MM is important for diagnosis, treatment and prognosis. Identification of SMM patients offer early treatment option which halts the progression of the disease thereby reducing the morbidity.

Immunophenotyping with multiparametric flow cytometry plays a significant role in SMM to determine prognosis and accurately distinguishing and quantitating neoplastic BMPCs.[8] Aberrant phenotype is defined by the absence of CD19 and/or CD45 expression, decreased expression of CD38, and overexpression of CD56 in neoplastic plasma cell.[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 which represents neoplastic plasma cell population in SMM; CD45 negativity is associated with poor clinical outcome. 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 and 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 diagnosis and monitoring of 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]

Baseline studies should include complete blood count, serum creatinine, serum calcium, skeletal survey, serum protein electrophoresis with immunofixation, serum FLC assay, bone marrow examination, and should include 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.[13]

At presentation, this case fulfilled the diagnostic criteria defined by Rajkumar, et al. for the diagnosis of SMM based on the results of the aforesaid examinations performed.[2] The prognostic influence of cytogenetic abnormalities was analyzed by Mayo Clinic group 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 was at low risk SMM.

4. Conclusion

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.

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

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 urine	Absent	
Serum immunoglobulin profile: IgG level IgM level IgA level	65.1gm/l < 0.17 gm/l <0.25gm/l	7.0 - 16.0 gm/l 0.4- 2.3gm/l 0.7- 4.0 gm/l
Urinary albumin: creatinine ratio	6.15mg/gm	<30.0mg/gm

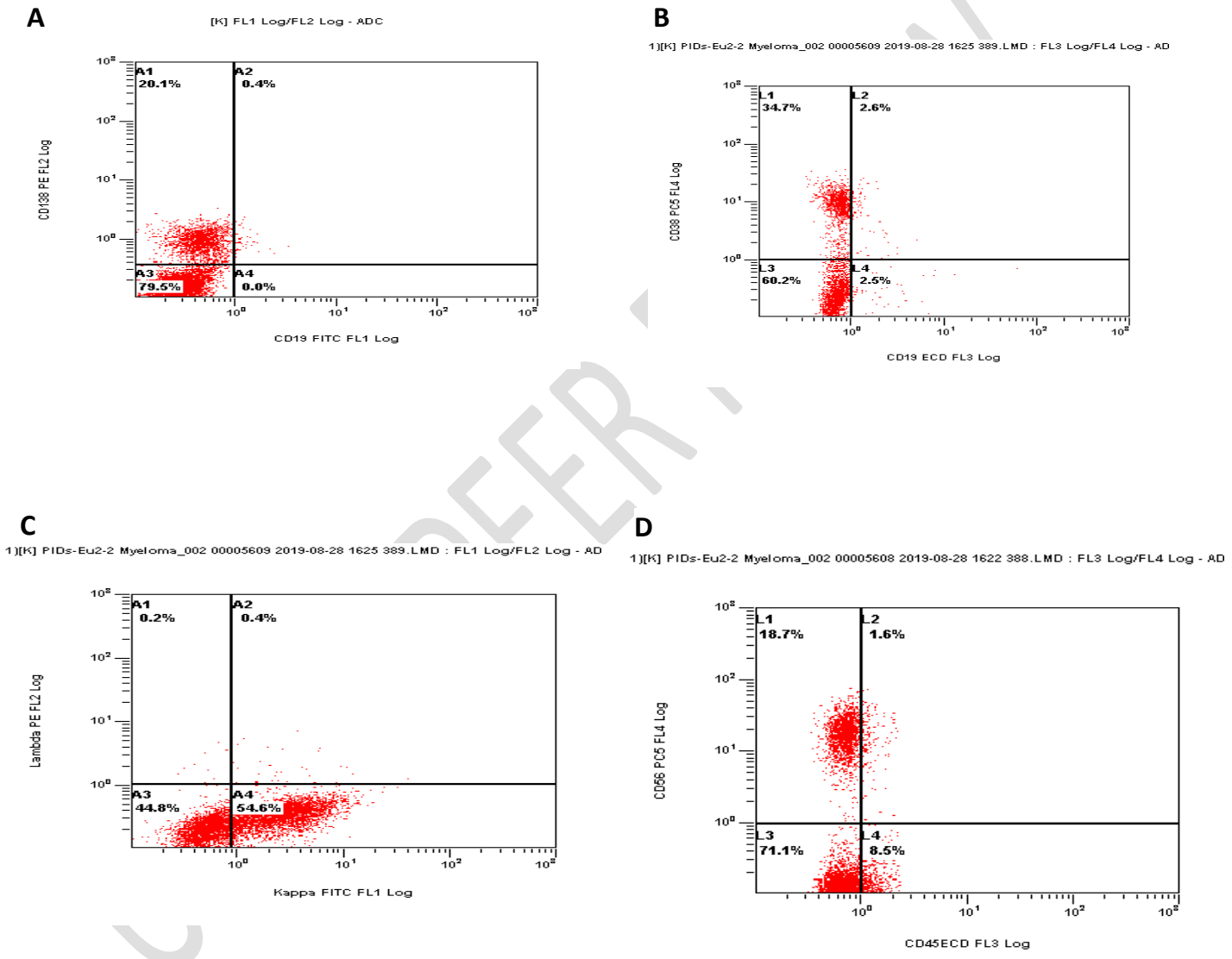

Figure 1: Flow cytometry immunophenotyping of bone marrow: Plasma cells (PCs) are first identified by gating using CD45, CD138 and CD38. **A** and **B**, CD138 (moderate) and CD38 (bright) expression identify plasma cell population with downregulation of CD19 (dim/-). **C** and **D**, Dim to moderate 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.

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