

# Two Cases of Multiple Myeloma Revealing 2 Clonal Plasma Cell Populations on Flowcytometry

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**Abstract:** Flowcytometric immunophenotyping is useful to identify clonal plasma cells which are helpful in the diagnosis of multiple myeloma. The standard diagnostic method involves the selection of abnormal plasma cells by CD38 and CD 138 which is generally positive. We present two cases who were diagnosed with multiple myeloma in the presence of lytic lesions in the skeletal survey and confirmed clonality on flowcytometry, revealing two populations of plasma cells with positive CD38 and negative CD138 and vice versa respectively.

**Keywords:** Multiple Myeloma, CRAB, Flowcytometry, Immunophenotyping, Paraprotein

## 1. Introduction

Multiple myeloma (MM) is a B-cell neoplasia, characterized by the clonal proliferation of malignant plasma cells in the bone marrow, the presence of monoclonal protein in the serum and/or urine, in symptomatic patients the presence of one or more CRAB features (elevated ionized calcium, renal failure, anaemia, and bone lesions) and proliferation of monoclonal plasma cells in the bone marrow. Flowcytometric immunophenotyping is used to identify neoplastic plasma cell populations in patients with multiple myeloma.

The standard diagnosis of multiple myeloma by flow cytometry is based on selection of population of CD38+/CD138+ positive cells. A number of previous studies have demonstrated that various antigens are expressed on plasma cells such as CD19, CD27, CD38, CD45, CD56, CD138, cyIg kappa and cyIg lambda.

Reactive plasma cells are characterized by low forward/side scatter (FSC/SSC) and high CD38 expression together with a CD19+/CD56- phenotype. In contrast, neoplastic plasma cells are CD19-/CD56+ or -, with high FSC/SSC and relatively low CD38 expression [2].

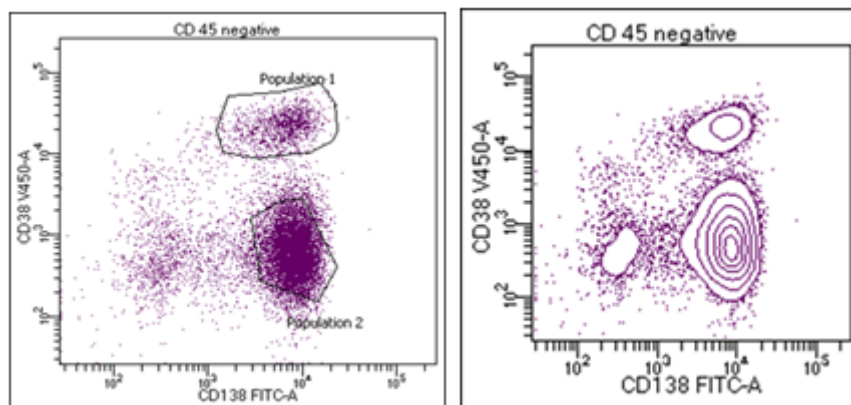
## 2. Case Report 01

A 76 - year - old female patient was admitted to a tertiary care teaching hospital with history of severe back pain and shoulder pain. Investigations revealed haemoglobin of 9.2 g/dl and ESR 120 mm/1st hour. Blood picture showed normochromic normocytic RBCs with marked rouleaux formation. WBCs were normal in number and showed a lymphocytic predominance with normal platelets.

Urine analysis showed proteinuria. Serum Creatinine was 1.2 mg/dl. Urine Bence - Jones protein was positive. Serum Protein Electrophoresis showed a monoclonal band in the gamma region, suggestive of a monoclonal gammopathy of 14 .4 g/L. Lytic lesions were seen in the skeletal survey. Ultra Sound Scan of abdomen was normal.

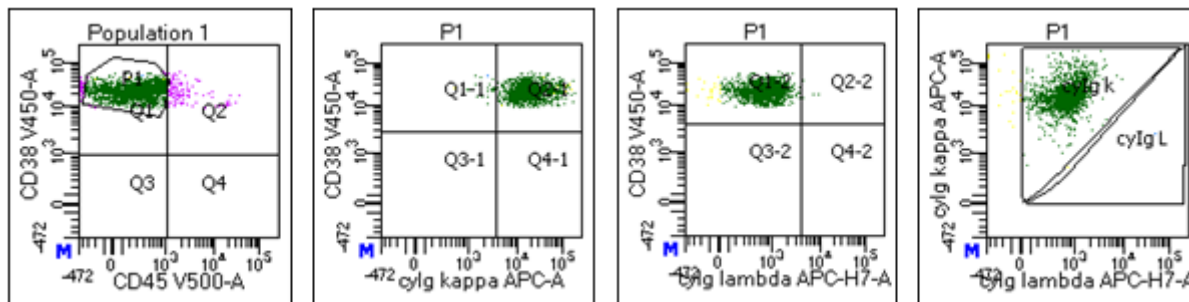
Bone marrow aspiration & biopsy showed approximately 40% of the nucleated marrow cells were a population of plasma cells, plasma blasts with a few bi-nucleated and multinucleated forms. Lymphocytes were normal. The findings of Bone marrow trephine biopsy were consistent with the bone marrow aspiration findings.

To confirm clonality of plasma cells, flowcytometry was performed by BD FACS Canto 2. Two populations of abnormal plasma cells were revealed.

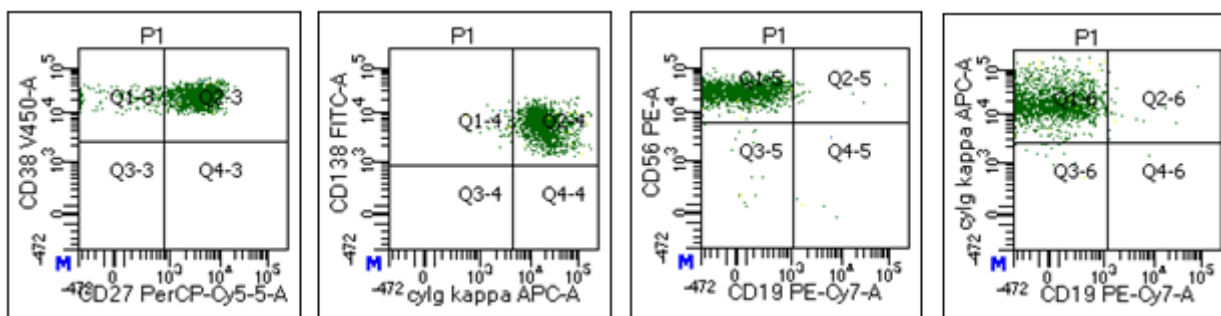


Population 1; This population was 3% and the results were bright positivity of CD38, CD138, CD56, CD27, cyIg

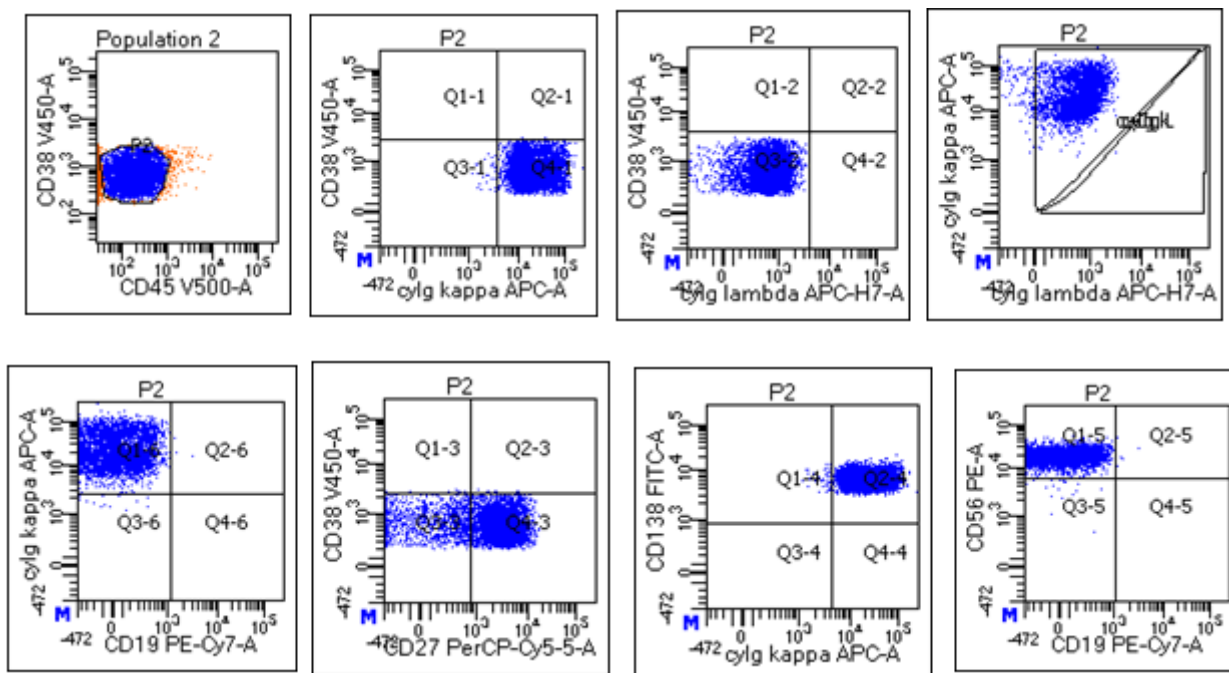
kappa with the negativity of CD 19, CD45 and cyIg lambda.



Population 2; This population was 14.8% and the results were bright positivity of CD 138, CD56, cyIg



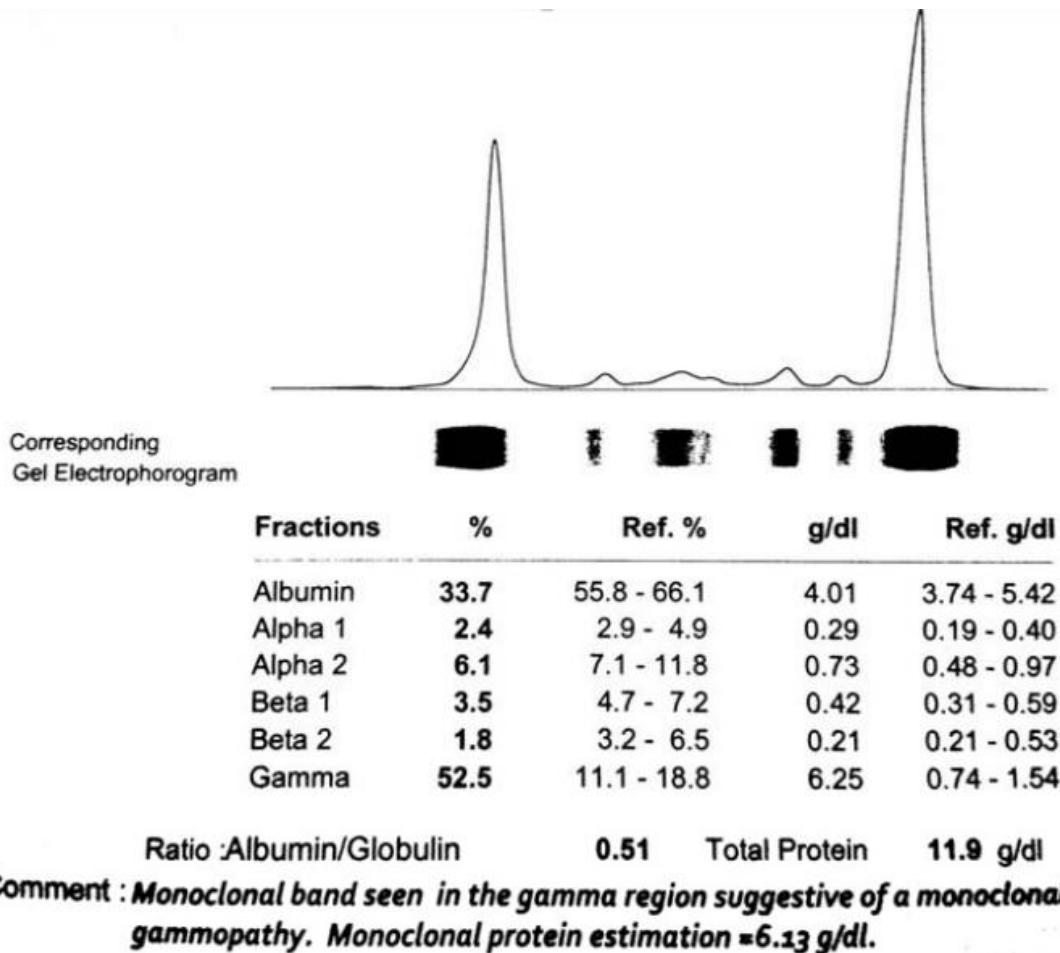
kappa and positivity of CD27 with the negativity of CD19, CD38, CD45 and cyIg lambda



### 3. Case Report 02

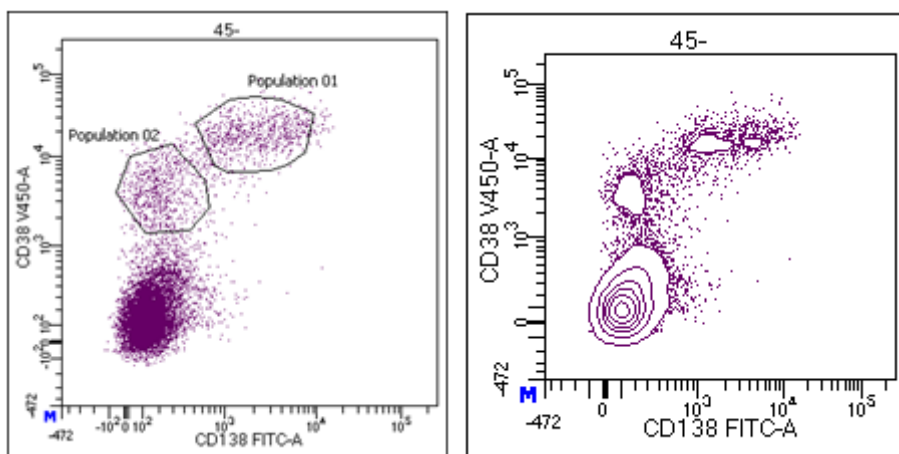
A 56 – year - old male patient was referred to a tertiary care teaching hospital with history of right hip pain radiating along the lower limb. Investigations revealed high ESR (120 mm/1<sup>st</sup> hour). Blood picture showed normochromic normocytic RBC and macrocytes with marked rouleaux formation and mild lymphocytic predominance with occasional plasmacytoid lymphocytes.

Urine analysis showed proteinuria. Serum creatinine was 144.2 µmol/L. Urine Bence – Jones protein was negative, Viral studies were negative. And he had normal serum electrolytes. Serum Protein Electrophoresis showed a monoclonal band in the gamma region, suggestive of a monoclonal gammopathy. Monoclonal protein estimation was 61.3 g/L



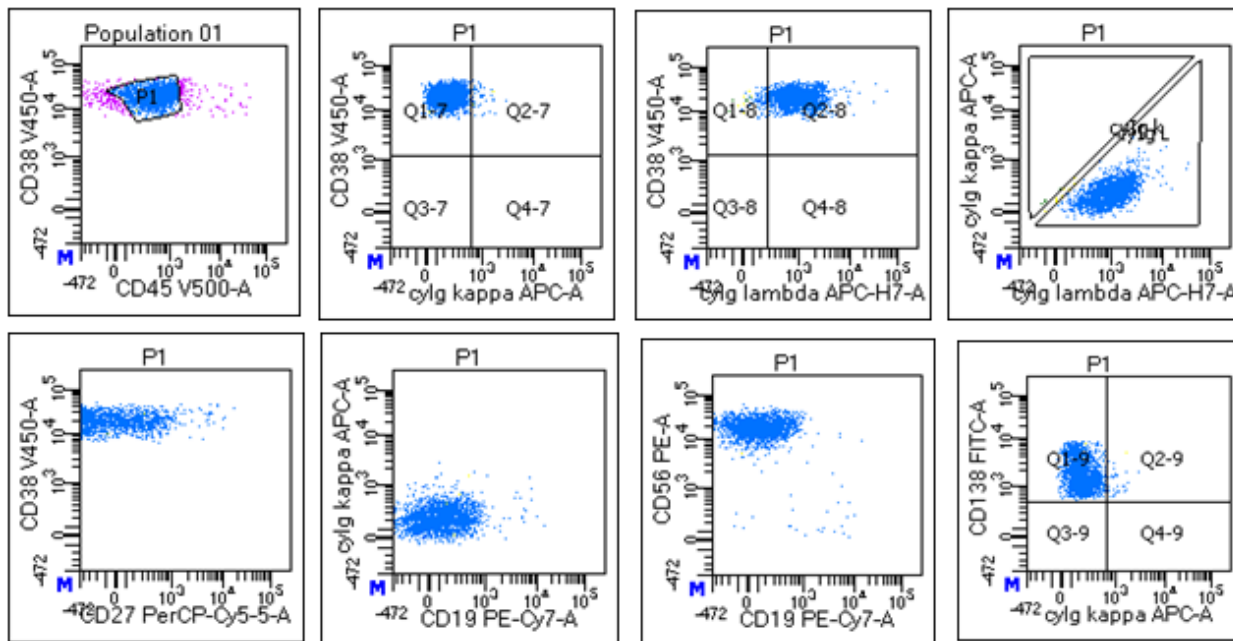
Lytic lesions were seen in the skeletal survey. Bone marrow aspiration biopsy showed increased plasma cells approximately 20% of the nucleated marrow cells with a few multinucleated forms. The findings of Bone marrow trephine biopsy were consistent with the aspiration findings.

To confirm clonality of plasma cells, flowcytometry was performed. Two populations of abnormal plasma cells were revealed.



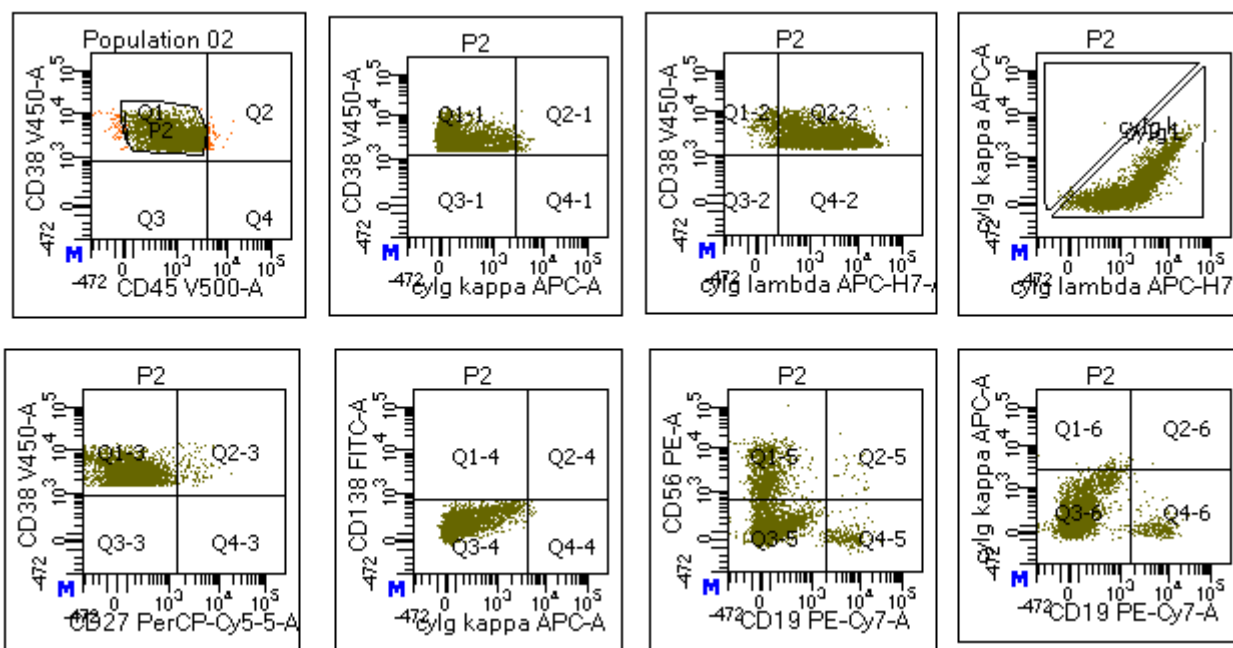
Population 1; This population was 4.6 % and the results were bright positivity of CD38, CD138, CD56, cyIg

lambda with the negativity of CD 19, CD27, CD45 and cyIg kappa.



Population 2; This population was 7.6 % and the results were bright positivity of CD38, cyIg lambda and dim CD

56 with the negativity of CD 138, CD 19, CD27, CD45 and cyIg kappa.



#### 4. Conclusion

In the presence of lytic lesions in the skeletal survey and confirmed clonality of plasma cells, Plasma cell myeloma/multiple myeloma were confirmed for both patients.

#### 5. Discussion

Flow cytometric immunophenotyping is used to identify neoplastic plasma cell populations in patients with multiple myeloma. Plasma cells represent the last stage of B-cell development. Maturation of B-lymphocytes toward plasma cells is a multistep process. During malignant transformation of plasma cells, the expression of these antigens may be altered. Both benign and clonal plasma

cells are usually positive for CD38, CD138, cytoplasmic light and heavy chain immunoglobulins.

The majority of true plasma cell neoplasm have abnormal surface immunophenotype, with decreased to absent CD 19, CD27, CD45 and occasionally decreased CD38, decreased or increased CD138[3].

Lack of expression of target molecules on tumor cells is an important resistance mechanism for antibody-based therapies. Patient NO 1 had negative CD38 expression (population 2) at diagnosis and so will not be a candidate for therapy such as Daratumumab. A minimum threshold of target antigen expression is required above which complement-mediated cytotoxicity can occur [4]. Therefore, we can believe that down regulation or loss

of CD38 might be an epigenetic “escape mechanism” of malignant plasma cells from antibody based treatments.

CD 45 marker is predominantly present in the early stages of Plasma cells development and decreases in expression with maturation. Presence of CD 45 negative Plasma cells in the bone marrow indicates late stage of the disease and appears to predict a less favorable prognosis. [6]

CD56 is expressed by ~ 70% of plasma cell myeloma and CD45 is negative in the majority of cases but occasional cases show dim or moderate CD45. Positivity of CD19 is seen in benign plasma cells and is negative in most plasma cell neoplasm [2]. CD 27 positivity of abnormal plasma cells in multiple myeloma is ~ 48% while it is positive in ~100% benign plasma cells [7].

CD138 (Syndecan-1) is a heparin sulphate proteoglycan responsible for growth factor binding, cell adhesion, apoptosis and control of myeloma growth [5]. CD 138 negative plasma cells are more primitive and have a higher proliferative potential than CD138 positive plasma cells. [8]

It is important to process the sample at the earliest possible, as the delay can cause a false negative CD138 expression. CD 138 has now been recommended as the gating marker for plasma cells along with CD38 indicating the importances that delay is avoided in all cases of plasma cell immunophenotyping [5].

Patients with low levels of CD138 (Patient 2) have a worse overall survival compared with high levels of CD138 in newly diagnosed, as well as in patients receiving high-dose chemotherapy followed by autologous Stem Cell Transplantation. These patients are also known to respond poorly to treatment with Lenalidomide [09].

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