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# The importance of flowcytometry study with the first aspirate taken during bone marrow aspiration in the diagnosis of multiple myeloma and follow-up of minimal residual disease

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## ABSTRACT

**Objective:** Flow cytometry (FC) is a diagnostic method supporting traditional morphological examination in disease follow-up and the diagnosis of Multiple myeloma (MM). Normal and atypical plasma cells (PCs) can be told apart from each other by means of FC method. The plasma cell rate is the highest in the blood obtained in the first aspirate during bone marrow aspiration in MM.

**Material and methods:** A total of 60 patients that have been diagnosed with MM between 2018 and 2020, including 30 patients whom flow cytometry was studied with the first aspirate during bone marrow aspiration, and 30 patients whom FC was studied with the second aspirate were included in our study. The characteristics of the patients were analyzed retrospectively from their files.

**Results:** The median ratio of plasma cells (PCs) detected by FC and bone marrow biopsy was 17,5% and 44%, respectively. While this rate was median 37,5% in patients that flow cytometric study was performed with the first aspirate, the rate was found to be median 7% in patients that FC was performed with the second sample. The PCs rates were statistically significantly higher with the flow cytometric study with the first aspirate than the second one (p=0.000).

**Conclusion:** Flow cytometric study with the first aspirate during bone marrow aspiration in patients with MM is diagnostically important.

**Keywords:** Multiple myeloma, flow cytometry, bone marrow aspiration, first aspirate

## INTRODUCTION

Multiple myeloma (MM) is a malignant neoplasm presenting with anemia, renal failure, and bone lesions due to the increase in atypical plasma cells in the bone marrow and the accumulation of excessive monoclonal proteins secreted from these cells in serum and urine and causing end organ damage (1). Biochemical parameters, immunofixation studies in serum and urine samples have a great place in the diagnosis of MM (1). Previously, the diagnosis of MM and the decision to start treatment was determined according to the CRAB (hypercalcemia, renal insufficiency, anemia, bone lesions) criteria, together with the bone marrow plasma cell ratio being > 10% (2). The International Myeloma Working Group (IMWG) revised these criteria in 2014 and added new biomarkers to the existing criteria that identify asymptomatic patients at high risk of progression (3). Among these criteria, clonal plasma cells (PC) in the bone marrow 60%, the ratio of free light chains (FLC) 100, and more than 1 focal lesion larger than 5 mm on MRI were accepted as treatment criteria. (3). The definitive diagnosis of MM is made by histopathological examination of the bone marrow biopsy material and microscopic examination of the bone marrow sample taken by bone marrow aspiration together with flow cytometric study (1).

Flowcytometry (FC) is a diagnostic method that supports traditional morphological examination in the diagnosis of MM and disease follow-up (4). With the FC method, normal and atypical plasma cells are distinguished from each other (4-6).

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It contributes to the evaluation of the possibility of progression to MM, one of the precursor forms that do not require treatment of FC, evaluation of the prognosis, the response to treatment and the minimal residual disease (MRD) (7-9).

Under normal conditions, bone marrow sample taken during bone marrow aspiration for diagnosis of hematological malignancies is aspirated in three stages. In the first stage, 0.5 cc of blood aspirated is spread on the slide and subjected to staining process and used in microscopic examination. Flowcytometric study is performed with 1-2 cc marrow sample taken in the second stage. 2-3 cc marrow sample taken at the last stage is sent to the laboratory for cytogenetic studies. The rate of PCs is highest in the blood taken at the first stage of MM. The proportion of PCs in the bone marrow sample taken in the later stages gradually decreases. This causes less malignant cells to be detected, especially in flowcytometric studies. This results in the detection of fewer PCs both at the time of diagnosis and during post-treatment bone marrow evaluation. Especially nowadays, MRD studies with FC have become more important.

In this study, we aimed to compare the PC rates of the patients who underwent flowcytometric study with the first marrow sample in the bone marrow aspiration performed at initial diagnosis and the PC rates of the patients who underwent flowcytometric study with the second sample and to examine the diagnostic importance.

## MATERIAL and METHODS

A total of 60 patients were included in our study, including 30 patients who were diagnosed with MM between 2018-2020, who underwent a flowcytometric study with the first marrow sample during bone marrow aspiration, and 30 patients who underwent flowcytometric study with the second bone marrow sample. The characteristics of the patients were analyzed retrospectively from their files. The sample taken for FC was taken with the first aspirate in the first group and with the second aspirate in the second group during aspiration.

Flowcytometry: Diagnosis with FC in MM and MRD evaluation depends on the detection of immunophenotypic abnormalities in malignant plasma cells rather than clonality evaluation with light chain analysis (10). Normal plasma cells express CD19, CD45, CD138 and bright CD38 and are negative for CD20 and CD56. In MM, PCs generally show abnormal CD56 expression and loss of CD19, and less frequently abnormal CD20, CD28 and CD117 expression (10-12). Panels recommended for disease monitoring in MM, therefore, minimally include the evaluation of CD19 and CD56, because abnormal expression of these antigens should identify neoplastic plasma cells at follow-up in at least 90% of patients with MRD (4, 13).

Analysis was performed on the bone marrow aspirates of patients with a diagnosis of MM or suspected of having MM who were sent for routine diagnostic analysis in our laboratory. Eight-color multiparametric was performed on bone marrow mononuclear cells isolated with the FC Ficoll gradient and stained with antibodies against CD45, CD19, CD38, CD56, CD138 and cytoplasmic kappa and lambda immunoglobulin light chains. Data were collected using BD FACSCanto II devices that collected 150,000 events; FC data were analyzed using BDFacs DIVA Software. Figure 1 shows the FC result of a patient diagnosed with MM.

Statistical Methods: Mean, standard deviation, median, minimum, maximum value frequency and percentage were used for descriptive statistics. The distribution of variables was checked with Kolmogorov-Simirnov test. Kruskal-Wallis and Mann-Whitney U test were used for the comparison of quantitative data. Wilcoxon test were used for the repeated measurement analysis. Chi-Square test was used for the comparison of the qualitative data. SPSS 27.0 was used for statistical analysis.

## RESULTS

Twenty-eight (46.7%) of the patients included in the study were female and 32 (53.3%) were male. The median age of the patients was 63 years. Median PC values determined by FC and bone marrow biopsy (BMB) at initial diagnosis, median biochemical results, median involvement with PET / CT, MM subtype, treatment preferences, (and autologous stem cell transplantation (ASCT) if it was performed) and the status of the patients at the last control are summarized in Table 1.

When all patients were evaluated, the median ratios of PCs detected by FC and BMB were 17.5% and 44%, respectively. While this ratio was 37.5% in patients who had FC with the first aspirate, the median was found to be 7% in patients who had FC with the second sample. The PC ratios of the patients who had FC with the first aspirate were statistically significantly higher than the PC ratios detected by FC with the second aspirate ($p = 0.000$). In both groups, the number of PC detected by BMB was higher than that those determined by FC (Figures 1 and 2.), but the PC ratios detected by BMB were similar to the PC ratios obtained by FC with the first aspirate (figure 2). However, the ratios of PC obtained by FC with the second aspirate was significantly lower than the PC ratios detected by BMB ($p < 0.05$) (Table 2). In both of the groups in which FC was performed with the first and second aspirate, the ratios of PC detected in BMB were 49.5% and 30%, respectively. There was no statistically significance ($p = 0.261$) (Table 2).

There was no statistically significant difference between patients with remission, refractory disease or with a mortal course in terms of patients' age ($p = 0.490$), gender distribution ($p = 0.087$), PC ratios by FC and BMB ($p = 0.078$ and $p = 0.829$, respectively), biochemical findings and PET / CT involvement. In the exitus group, the first sample PC ratio by FC was significantly lower than the remission and refractory groups ($p = 0.003$). In the group with remission, the first sample PC by FC ratio was significantly lower than the refractory group ($p < 0.05$). The lines of treatments in the refractory group were significantly higher than in the exitus and remission group ($p = 0.019$). The proportion of patients who underwent ASCT in the remission group was significantly higher than the exitus and refractory groups ($p = 0.000$). The proportion of patients who underwent ASCT did not differ significantly ($p > 0.05$) between the exitus and refractory groups (Table 3).

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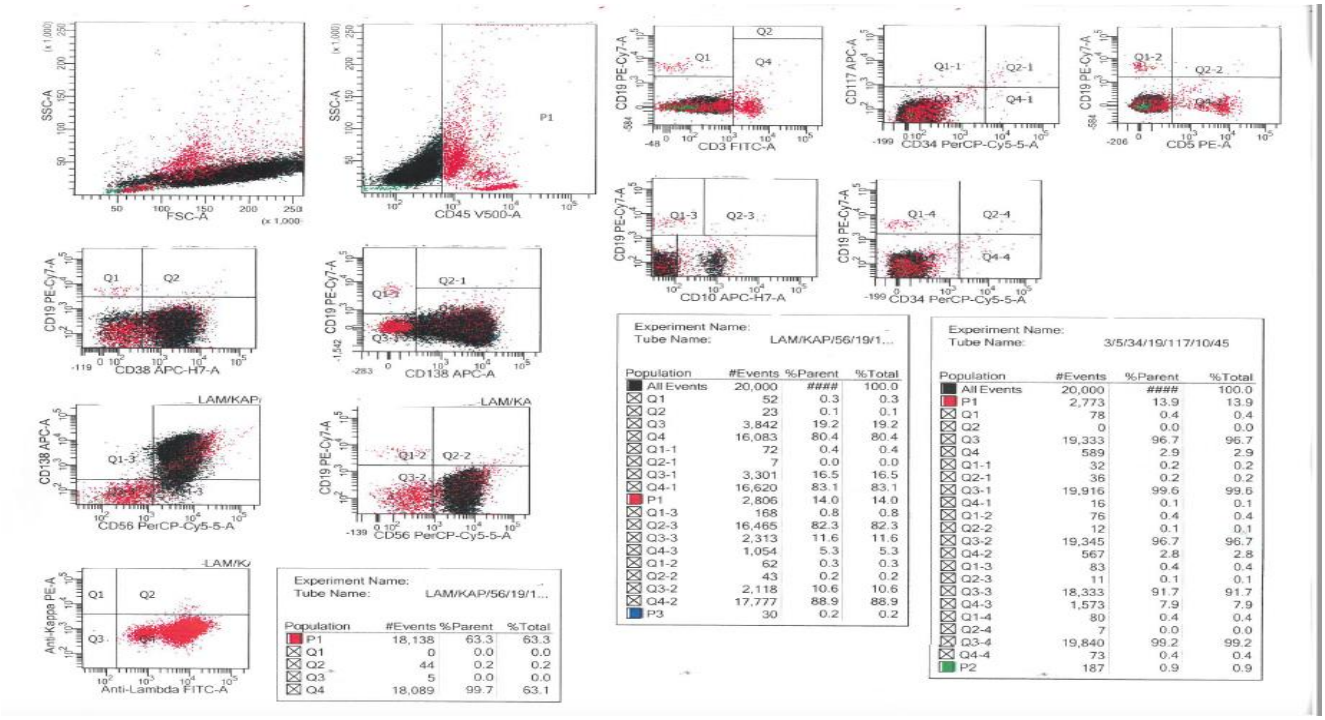


**Figure 1:** FC result of a MM patient (Analysis on all cells revealed 83.1% abnormal clonal plasma cell population showing CD45-, CD117-, CD38+, CD138+, CD56+, CD19-, Anti-Lambda + immunophentotypic features. These findings are consistent with MM).

**Table 1:** Demographic characteristics of patients at the time of diagnosis

| | | Min-Max | | | Median | Mean±sd/n-% | | |
|---|---|---|---|---|---|---|---|---|
| Age | | 40,0 | - | 85,0 | 63,0 | 63,4 | ± | 10,3 |
| Gender | Female | | | | | 28 | | 46,7% |
| | Male | | | | | 32 | | 53,3% |
| Aspiration | First aspirate | | | | | 30 | | 50,0% |
| | Second aspirate | | | | | 30 | | 50,0% |
| PC ratio by FC | | 1,2 | - | 83,0 | 17,5 | 24,8 | ± | 21,2 |
| PC ratio by BMB | | 10,0 | - | 90,0 | 44,0 | 46,9 | ± | 21,1 |
| Hb | | 5,0 | - | 16,0 | 9,9 | 9,9 | ± | 2,0 |
| Creatinin | | 0,5 | - | 6,8 | 1,3 | 2,1 | ± | 1,8 |
| Ca | | 7,4 | - | 16,0 | 9,7 | 10,1 | ± | 2,0 |
| LDH | | 100 | - | 760 | 188 | 214 | ± | 110 |
| PET/CT, SUV-MAX | | 2,0 | - | 14,5 | 5,1 | 6,3 | ± | 3,2 |
| ***MM Type*** | | | | | | | | |
| Ig A Kappa | | | | | | 3 | | 5% |
| Ig A Lambda | | | | | | 3 | | 5% |
| Ig G Kappa | | | | | | 20 | | 33,3% |
| Ig G Lambda | | | | | | 7 | | 11,7% |
| Kappa light chain | | | | | | 19 | | 31,7% |
| Lambda light chain | | | | | | 8 | | 13,3% |
| ***Treatment*** | | | | | | | | |
| VEL-DEX | | | | | | 1 | | 1,7% |
| VCD | | | | | | 44 | | 73,3% |
| VCD/RD | | | | | | 6 | | 10,0% |
| VCD/VRD | | | | | | 5 | | 8,3% |
| VCD/VRD-KRD | | | | | | 2 | | 3,3% |
| VCD/VRD/VRD+DARA | | | | | | 1 | | 1,7% |
| VEL-DEX/RD | | | | | | 1 | | 1,7% |
| Treatment line | I | | | | | 44 | | 73,3% |
| | II | | | | | 13 | | 21,7% |
| | III | | | | | 3 | | 5,0% |
| ASCT | (-) | | | | | 30 | | 50,0% |
| | (+) | | | | | 30 | | 50,0% |
| Remission | | | | | | 38 | | 63,3% |
| Refractory | | | | | | 10 | | 16,7% |
| Exitus | | | | | | 12 | | 20,0% |

**PC: Plasma cell, FC: Flow cytometry, BMB: Bone marrow biopsy, Hb: Hemoglobin, LDH: Lactate dehydrogenase, PET/CT: Positron emission tomography/computorised tomography, Ig: Immunglobulin, VEL-DEX: Bortezomib-Dexamethasone, VCD: Bortezomib-Cyclophosphamide-dexhamethasone, RD: Lenalidomide-Dexhamethasone, VRD: Bortezomib, lenalidomide-dexhamethasone, DARA: daratumumab, ASCT: Autologous stem cell transplantation**

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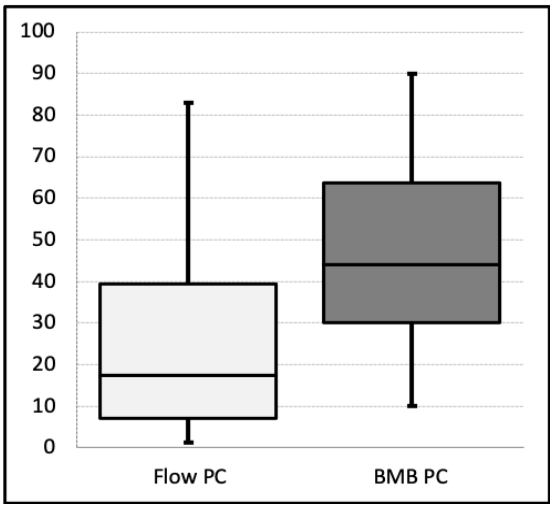


**Figure 2:** Comparison of plasma cell ratios determined by bone marrow biopsy and flow cytometry in all patients

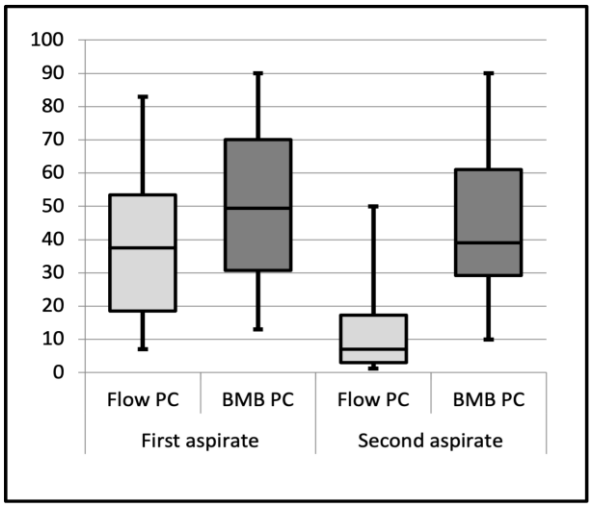


**Figure 3:** Comparison of plasma cell ratios of the patients whom FC was studied with the first aspirate and the plasma cell ratios of the patients whom FC was studied with the second aspirate

**Table 2:** Differences of FC study done with first aspirate and second aspirate

| | First aspirate | | Second aspirate | | p | |
|---|---|---|---|---|---|---|
| | Mean±sd | Median | Mean±sd | Median | | |
| PC ratio by FC | 37,93 ± 20,25 | 37,50 | 11,68 ± 12,07 | 7,00 | ***0,000*** | m |
| PC ratio by BMB | 50,07 ± 21,59 | 49,50 | 43,68 ± 20,43 | 39,00 | 0,261 | m |
| Difference | 12,13 ± 10,61 | 10,50 | 32,00 ± 16,99 | 30,50 | ***0,000*** | m |
| Intra Group Difference | ***0,000*** | w | ***0,000*** | w | | |

PC: Plasma cell, FC: Flowcytometry, BMB: Bone marrow biopsy, [m] Mann-whitney u test/ [w] Wilcoxon test

**Table 3:** FC and BMB and PC ratios in remission, retractory and exitus groups

| | Remission | | Refractory | | Exitus | | p | |
|---|---|---|---|---|---|---|---|---|
| | Mean±sd/n-% | Median | Mean±sd/n-% | Median | Mean±sd/n-% | Median | | |
| Age | 63,68 ± 10,15 | 63,00 | 60,67 ± 11,98 | 60,00 | 64,33 ± 10,20 | 68,50 | 0,490 | K |
| Gender Female | 18 47,4% | | 4 40,0% | | 6 50,0% | | 0,887 | $X^2$ |
| Male | 20 52,6% | | 6 60,0% | | 6 50,0% | | | |
| PC by FC | 24,54 ± 23,02 | 15,00 | 34,30 ± 18,18 | 33,00 | 17,73 ± 14,64 | 14,00 | 0,078 | K |
| PC by BMB | 45,14 ± 23,56 | 35,50 | 45,30 ± 18,30 | 42,00 | 53,67 ± 13,47 | 48,00 | 0,829 | K |
| Hb | 9,80 ± 2,17 | 9,55 | 10,57 ± 1,44 | 10,30 | 9,49 ± 1,82 | 9,30 | 0,182 | K |
| Creatinin | 1,92 ± 1,71 | 1,20 | 2,31 ± 2,08 | 1,20 | 2,60 ± 2,09 | 1,90 | 0,849 | K |
| Ca | 10,14 ± 2,07 | 9,50 | 8,99 ± 1,11 | 9,35 | 10,66 ± 2,09 | 10,00 | 0,199 | K |
| LDH | 204,4 ± 96,1 | 177,0 | 210,8 ± 63,2 | 203,5 | 244,6 ± 171,2 | 223,5 | 0,328 | K |
| PET/CT SUVMAX | 6,28 ± 3,26 | 5,30 | 6,47 ± 2,90 | 5,85 | 6,43 ± 3,73 | 4,95 | 0,790 | K |

[K] Kruskal-wallis (Mann-whitney u test) / [$X^2$] Chi-sqaure test, PC: Plasma cell, FC: Flow cytometry, BMB: Bone marrow biopsy, Hb: Hemoglobin, LDH: Lactate dehydrogenase, PET/CT: Positron emissiom tomography/computorised tomography, ASCT: Autologous stem cell transplantation

# DISCUSSION

Today, FC is an indispensable method in the diagnosis, treatment and follow-up of plasma cell neoplasms (PCN) together with histopathological and biochemical analyzes. In order to examine PCNs using FC, it is necessary to distinguish the immunophenotype of normal and neoplastic plasma cells (**14**). Numerous studies have been reported on the immunophenotype of PCs and the immunophenotype of polyclonal PCs (**4,7,15-17**). In addition, in order to determine the effectiveness of the treatment, the detection of MRD by FC method has been investigated in several studies (**13,18,19**).

The place of FC in the diagnosis of MM and follow-up of MRD is indisputable. Our aim in this study is not only to reinforce the importance of FC in the diagnosis of MM, but also to determine the contribution of FC with the first marrow sample taken during bone marrow aspiration. To best of our knowledge, our study is first in this field.

Pavia B. et al. revealed that FC and MRD monitoring is one of the most important prognostic factors in elderly MM patients, and cytogenetic risk is complementary and superior to traditional response criteria (**20**).

Cannizzo et al. showed that FC correlates with histopathological studies (14). In our study, PC ratios in patients who had FC with the first aspirate were similar to PC ratios determined by BMB. However, we found that the PC ratio was statistically significantly lower in patients who had FC with the second aspirate compared to BMB. While the median PC ratio was 37.5% in the patients whom FC was studied with the first aspirate, it was found 7% those studied with the second aspirate. In samples obtained by BMB, they were found to be 49.5% and 39% in both groups, respectively.

Nadav et al. showed that FC in MM significantly underestimated the number of PCs compared to counting PCs in aspirate smears. They also provided evidence suggesting that the reason for this underestimation was that FC samples lacked spicules to which MM cells were attached (21).

Terpstra et al. showed that PC was lower in aspirate smears when bone marrow aspirate smears were compared with BMB (22). In our study, the ratios of PCs by FC were found to be lower than BMB in both groups. However, although PC ratio was lower in first aspirate FC results than BMB, it was not statistically significant. The ratio of PC obtained in FC with the second aspirate was statistically lower than the ratio of PC detected by BMB ($p < 0.05$).

Ely S. et al. showed that in MM, their PCs bind strongly to other MM cells and stroma, as they contain large amounts of adhesion factors such as CD138 and CD56 (23).

Probably for this reason, some of the PC's cannot be detected in the aspiration material and less PC is detected in FC compared to BMB. Therefore, it is very important to perform a FC with the first sample during bone marrow aspiration. Because, when the FC is not performed with the first aspirate, the marrow sample becomes diluted at every stage. In addition, the adhesion property of PCs increases in the invitro environment. As a result, the PC ratio is detected lower than expected, which reduces the diagnostic efficiency of FC.

## CONCLUSION

Flowcytometric studies are an indispensable diagnostic method in addition to histopathological and biochemical studies in the diagnosis of MM. It has also proven its importance in MRD follow-up. In our study, we aimed to show that performing a FC with the first aspirate sample contributes to the diagnosis. However, prospective studies are needed in larger patient groups.

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**Ethical issues:** Ethics approval for the study was obtained from Istanbul Yeni Yuzyıl University Non-Interventional Ethics Committee, Date: 05.04.2021, Number: 2021/04-636

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