İstanbul Med J 2023; 24(3): 251-5
**DOI:** 10.4274/imj.galenos.2023.26675

# Accuracy of Blast Enumeration in Bone Marrow by Cytomorphology, Flow Cytometry Immunophenotyping, and Immunohistochemistry Methods in Patients with Myelodysplastic Neoplasm and Acute Myeloid Leukemia

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## ABSTRACT

**Introduction:** Blast percentage (BP) of bone marrow (BM) is crucial for diagnosis, classification, and prognosis in patients with myelodysplastic neoplasms (MDS) and acute myeloid leukemia (AML). Cytomorphology (CM) is considered the gold standard for BM blast counts. Immunohistochemistry (IHC) and flow cytometry immunophenotyping (FCI) methods are used as additional tools. This study aims to compare the overall accuracy of CM, FCI, and IHC in determining BM BP and to evaluate the accuracy of diagnostic and prognostic BP groups determined by these methods in MDS and AML cases.

**Methods:** CM, CD34-IHC, and FCI were performed on BM biopsy and aspiration samples from MDS and AML patients diagnosed between 9/2019 and 6/2021. Based on BP, cases were divided into four groups: <5%, ≥5%-<10%, ≥10%-<20%, ≥20%. The accuracy of the three methods was statistically compared in terms of blast values and BP groups.

**Results:** Sixty-eight cases were analyzed. The Pearson-r correlation coefficients for FCI-IHC, CM-FCI, and CM-IHC comparisons were 0.8865, 0.8787, and 0.9670, respectively, suggesting a strong correlation. A comparison of BP groups revealed 86.7%, 79.4%, and 88.2% of the cases in the same blast range by CM-FCI, CM-IHC and IHC-FCI, respectively. Fourteen (20.6%) cases were placed in two different BP groups using different methods.

**Conclusion:** There was a strong correlation between CM, FCI, and IHC in determining BP; however, one-fifth of the cases were classified differently by three methods, leading to diagnostic and prognostic changes. It is most reliable to combine all three methods taking into account the advantages and disadvantages of each.

**Keywords:** Bone marrow, blast, cytomorphology, flow cytometry, immunohistochemistry, CD34

## Introduction

Myelodysplastic neoplasms (MDS) are a group of clonal hematopoietic stem cell neoplasms defined by cytopenias and morphologic dysplasia characterized by progressively ineffective hematopoiesis and increased risk of acute myeloid leukemia (AML) (1). The term "myelodysplastic syndromes", which was being used in the former editions of World Health Organization (WHO) classifications, has been replaced by "MDS" in the latest edition, which was published in 2022 (1). The annual age-adjusted incidence is approximately 4.0/100,000, and the incidence increases with age (2). Survival is highly variable in MDS patients (3).

Over the years, several classification systems for predicting the prognosis, therapy response, and transformation to AML have been proposed, including the International Prognostic Scoring System (IPSS) and revised IPSS, with revised IPSS being the most up-to-date and widely used (4-7). Studies on large numbers of MDS patients indicated that 34-39% of the cases present with higher risk disease (1,7). The prognostic factors scored in the revised IPSS are the percentage of blast cells in the bone marrow (BM), cytogenetics, hemoglobin concentration, platelet count (blood) and absolute neutrophil count (blood) (7). Thus, the detection of blast percentage (BP) in the BM is crucial in diagnosing AML but also critical in the prognostic classification of MDS.

Three methods can be used to determine the BP in BM: cytomorphology (CM), flow cytometry immunophenotyping (FCI), and immunohistochemical (IHC) examination. In the most recent WHO classification, CM is considered the gold standard for blast counts (1). To

*The manuscript has been presented as oral presentation in 34th European Congress of Pathology held in Basel, Switzerland in 3-7 September 2022.

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**Cite this article as:** Hacıhasanoğlu E, Özkan F. Accuracy of Blast Enumeration in Bone Marrow by Cytomorphology, Flow Cytometry Immunophenotyping, and Immunohistochemistry Methods in Patients with Myelodysplastic Neoplasm and Acute Myeloid Leukemia. İstanbul Med J 2023; 24(3): 251-5.

**Received:** 20.03.2023
**Accepted:** 23.07.2023

determine the BP in BM, it is recommended that a 500-cell differential count of all nucleated cells be performed in a smear or trephine biopsy imprint (1). However, blast counts may be hard to appreciate in CM due to low cellularity, BM fibrosis, poor preparation quality, or low observer experience (8-12). Therefore, CD34 IHC analysis is recommended as a method for evaluating the BP in MDS cases (8,13). Other than that, studies report a strong correlation between FCI and CM and state that the addition of FCI to the diagnostic work-up can provide increased accuracy and reproducibility (14-16).

The objective of this study was to evaluate the accuracy of BM BP determined by CM, FCI, and IHC, as well as to evaluate the accuracy of these three methods for diagnosing and predicting BP in cases of AML and myelodysplastic syndrome.

## Methods

### Case Selection

This study is designed as a retrospective study performed by evaluating BM material including BM trephine biopsy, BM aspirates, and BM aspirate smears of MDS cases, AML cases, and cases diagnosed with AML and receiving bone marrow transplantation (BMT) between 9/2019 and 6/2021 in a single center. For inclusion in the study, BM aspiration samples should be analyzed by CM and FCI, and CD34 IHC should be applied to BM biopsy samples. Regardless of the French-American-British (FAB) classification, all AML cases fulfilling the study inclusion criteria were included in the study. A case lacking at least one of these three studies was excluded from the study. Also, cases with a diagnosis of AML but without CD34-positive blasts were not included in the study. Figure 1 shows the flowchart of the study inclusion and the exclusion process. Ethical approval for this study was obtained from the Yeditepe University Non-Interventional Clinical Studies Ethics Committee (decision number: E.83321821-805.02.03-147, date: 10.02.2023). The study protocol conformed to STARD and ethical guidelines established in the World Medical Association Declaration of Helsinki.

### The Evaluation of Cases

In each case; BP was evaluated by CM, IHC, and FCI. Grade of BM fibrosis according to the European Consensus was documented for each case (17). CM was evaluated by a single pathologist. CD34-positive BP was determined by the IHC method in BM trephine biopsies in all cases. CD34 immunohistochemical staining (clone QBEnd/10, Leica Biosystems) was applied to formalin-fixed-paraffin-embedded tissues using an automated staining device (Leica ST5010 Autostainer XL, Leica Biosystems). The percentage of CD34-positive blasts was determined by the FCI method in all cases of BM aspiration. All aspiration samples were processed within 2 hours. Flow cytometry analysis was done with Beckman Coulter Cytomics NAVIOS system and List Mode analysis was done with KALUZA Software Analysis program. According to BP, cases were divided into four groups: <5%, ≥5%-<10%, ≥10%-<20%, ≥20%.

### Statistical Analysis

Pearson's correlation coefficient and Spearman's rank correlation coefficient were used to assess the correlation between blast cell percentages determined by the CM, IHC and FCI methods. The accuracy

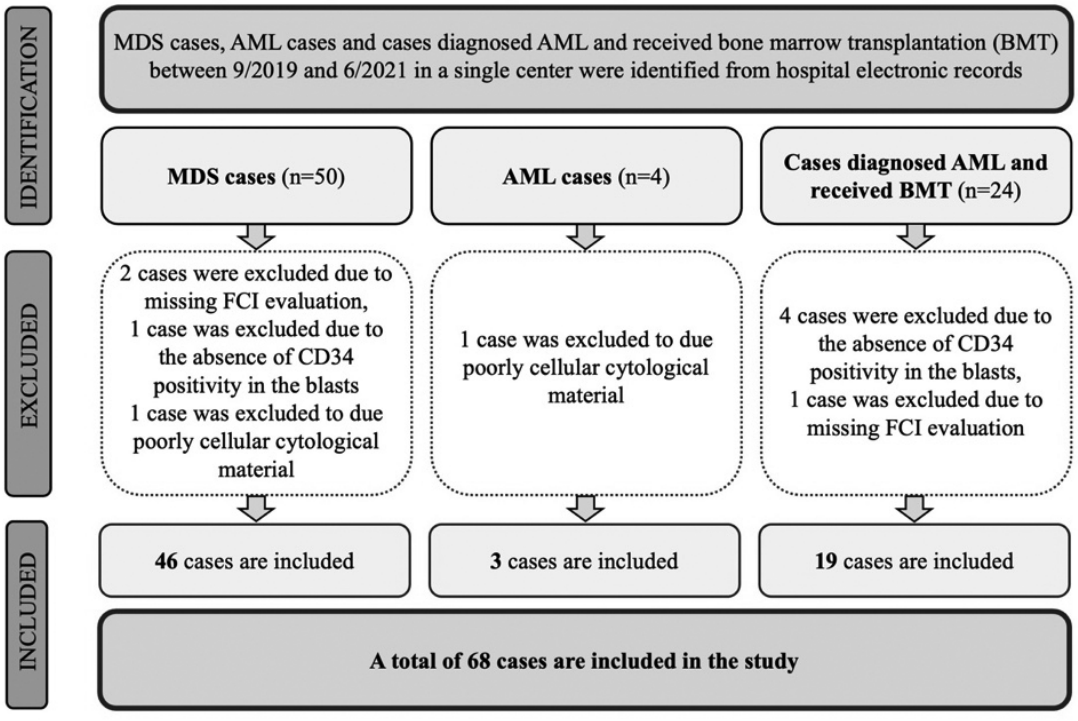


**Figure 1.** Flowchart of case selection
MDS: Myelodysplastic neoplasms, AML: Acute myeloid leukemia, FCI: Flow cytometry immunophenotyping

of the BP groups determined by each method was compared. The effect of BM fibrosis on the determination of BP groups using different methods was evaluated by Mann-Whitney U test. The statistical significance level was taken as 5% in the calculations. The analysis was conducted using the SPSS (IBM SPSS for Windows, versiyon 26) statistical package program.

## Results

A total of 68 cases were analyzed. The cases were composed of 39 MDS, 7 MDS-EB, 3 AML, and 19 cases of AML treated by BMT. Among 22 patients diagnosed with AML, the FAB classification was as follows: 6 AML-M1, 11 AML-M2, 2 AML-M3, 2 AML-M4 and 1 AML-M5. The mean age was 64.6 in MDS cases and 51.2 in AML cases. The female to male ratio was 1.3:1.

In the CM-FCI, CM-IHC, and FCI-IHC comparisons, the Pearson correlation coefficients were 0.8865, 0.8787, and 0.9670, respectively, suggesting a strong correlation. A comparison of CM-FCI cases based on BP groups revealed that 86.7% were in the same blast range (Table 1). Based on the CM-IHC comparison, 79.4% of the cases falls within the same blast range (Table 2). There was an 88.2% correlation between IHC-FCI BP groups based on comparison of the two methods (Table 3). In 79.4% (n=54) of the cases, the BP group determined by all three methods was the same. The remaining 20.6% (n=14) of the cases were placed in two different BP groups using different methods. In these 14 cases, BP groups determined by different methods were consecutive groups. Compared with CM, 5 of these cases (7.4% of all cases) were moved to one upper BP group with either IHC or FCI, whereas 9 cases (13.2% of all cases) were classified in one lower BP group. None of the cases were placed in three different BP groups using three different methods.

BM fibrosis was evaluated in a two-tiered fashion: grade 0-1 and grade 2-3. The cases that were in the same prognostic group by every three methods (n=54) and the cases that were classified differently by different methods (n=14) did not have a significant difference in terms of the presence of marrow fibrosis. The BM fibrosis grades in the first group were as follows: grade 0: 4 cases, grade 1: 12 cases, grade 2: 27 cases, and grade 3: 11 cases. In the second group, the BM fibrosis grades were grade 0: 1 case, grade 1: 3 case, grade 2: 7 case, and grade 3: 3 case. The grade of BM fibrosis did not affect the determination of prognostic groups using different methods (Table 4).

## Discussion

Detection of blast rate in the BM is important in the determination of MDS subgroups and in the differential diagnosis of MDS-AML. Also,

**Table 1. Comparison of blast percentage groups by CM and FCI**

| | CD34-positive blasts by FCI | | | | |
|---|---|---|---|---|---|
| **Blasts by CM** | **<5%** | **≥5%-<10%** | **≥10%-<20%** | **≥20%** | **Total** |
| <5% | 53 (77.9%) | 1 (1.5%) | 0 (0%) | 0 (0%) | 54 (79.4%) |
| ≥5%-<10% | 4 (5.9%) | 1 (1.5%) | 0 (0%) | 0 (0%) | 5 (7.4%) |
| ≥10%-<20% | 0 (0%) | 1 (1.5%) | 1 (1.5%) | 0 (0%) | 2 (2.9%) |
| ≥20% | 0 (0%) | 1 (1.5%) | 2 (2.9%) | 4 (5.9%) | 7 (10.3%) |
| Total | 57 (83.8%) | 4 (5.9%) | 3 (4.4%) | 4 (5.9%) | 68 (100%) |

CM: Cytomorphology, FCI: Flow cytometry immunophenotyping

**Table 2. Comparison of blast percentage groups by CM and IHC**

| | CD34-positive blasts by IHC | | | | |
|---|---|---|---|---|---|
| **Blasts by CM** | **<5%** | **≥5%-<10%** | **≥10%-<20%** | **≥20%** | **Total** |
| <5% | 50 (73.5%) | 4 (5.9%) | 0 (0%) | 0 (0%) | 54 (79.4%) |
| ≥5%-<10% | 5 (7.4%) | 0 (0%) | 0 (0%) | 0 (0%) | 5 (7.4%) |
| ≥10%-<20% | 0 (0%) | 2 (3%) | 0 (0%) | 0 (0%) | 2 (3%) |
| ≥20% | 0 (0%) | 0 (0%) | 3 (4.4%) | 4 (5.9%) | 7 (10.3%) |
| Total | 55 (80.9%) | 6 (8.8%) | 3 (4.4%) | 4 (5.9%) | 68 (100%) |

CM: Cytomorphology, IHC: Immunohistochemistry

**Table 3. Comparison of blast percentage groups by IHC and FCI**

| | CD34-positive blasts by FCI | | | | |
|---|---|---|---|---|---|
| **CD34-positive blasts by IHC** | **<%5** | **≥5%-<10%** | **≥10%-<20%** | **≥20%** | **Total** |
| <5% | 53 (77.9%) | 2 (2.9%) | 0 (0%) | 0 (0%) | 55 (80.8%) |
| ≥5%-<10% | 4 (5.9%) | 1 (1.5%) | 1 (1.5%) | 0 (0%) | 6 (8.9%) |
| ≥10%-<20% | 0 (0%) | 1 (1.5%) | 2 (2.9%) | 0 (0%) | 3 (4.4%) |
| ≥20% | 0 (0%) | 0 (0%) | 0 (0%) | 4 (5.9%) | 4 (5.9%) |
| Total | 57 (83.8%) | 4 (5.9%) | 3 (4.4%) | 4 (5.9%) | 68 (100%) |

IHC: Immunohistochemistry, FCI: Flow cytometry immunophenotyping

**Table 4. Comparison of the BM fibrosis grades of the cases that are placed in the same BP group by three methods and those that are placed in different BP groups by different methods**

| BM fibrosis grade | Cases placed in the same blast percentage group using three methods (n=54) (%) | Cases placed in different blast percentage groups using different methods (n=14) (%) |
|---|---|---|
| Grade 0 | 4 (7.5%) | 1 (7.2%) |
| Grade 1 | 12 (22.2%) | 3 (21.4%) |
| Grade 2 | 27 (50%) | 7 (50%) |
| Grade 3 | 11 (20.3%) | 3 (21.4%) |

BM: Bone marrow, BP: Blast percentage

percentage of blast cells in the BM is among the prognostic factors scored in the revised IPSS, a standard for assessing the prognosis of primary untreated adult patients with MDS (7). This increases the importance of accurately determining the blast rate in the BM.

In this study, a strong correlation was observed for absolute values of blasts with CM, IHC, and FCI evaluation methods. Almost 80% of the cases were placed in the same BP group using three methods. However, in 20% of the cases, the BP group was determined differently by different methods, leading to a change in the diagnostic and prognostic blast group of the cases. BM fibrosis grade did not show any effect in determining the prognostic groups by different methods.

CM is considered the gold standard for blast counts in BM. Cytomorphological assessment relies heavily on the quality of the cytological smears and the experience of the observer, which makes the reproducibility of this method limited in some circumstances. Low cellularity, BM fibrosis, poor preparation quality, and low observer experience have been shown to have an effect on cytomorphological blast counts (8-12). CM is shown to be a reliable method in blast counting with respect to the 5% cut-off; however, the reproducibility of the blast counts decreases under the 5% limit, especially under 2% (10,11). Also, it has been reported that smears taken from patients with hypoplastic MDS or MDS with fibrosis greater than WHO grade 2 can show low cellularity, and the blast count may not be fully representative in these cases (8,18,19).

The drawbacks of CM in determining blast cell percentage make immunohistochemical analysis and flow cytometry analysis with CD34 especially helpful when there is hypocellular BM or fibrosis, which often causes underestimation of BP in smears (13). Another important point is identifying CD34-positive cell clusters by IHC in the BM, which are reported to have prognostic importance for progression to AML (20,21). The European LeukemiaNet recommends the evaluation of CD34-positive cells in BM biopsy as a standard diagnostic tool in the approach to MDS (22). As for the use of FCI in the evaluation of BP in BM, while some studies show that FCI detects a lower number of blasts than CM (23,24), newer studies report a strong correlation between FCI and CM (14-16). In their multicenter study, Font et al. (25) investigated the reproducibility of CD34-positive cell count by FCI. They found an overall excellent agreement on CD34-positive cell count among participants and concluded that the standardization of routine flow cytometry laboratory practices is mandatory (25). In a more recent study, Johansson et al. (26) reported excellent concordance between seven cytometrists in myeloid progenitor cell count, concluding that FCI offers a reliable diagnostic and prognostic measurement in MDS patients.

Previous studies have shown varying results regarding the correlations between CM-IHC and CM-FCI. Hodes et al. (27) assessed the accuracy of blast cell quantification in 16 BM samples containing varying numbers of CD34-positive blasts. There was a poor correlation ($R^2$=0.52) between CM BP and trephine biopsy BP stained with CD34. A good correlation was demonstrated between the number of blasts determined by FCI and IHC ($R^2$=0.81). Recent research by Saft et al. (28) determined CD34+ blast counts in 132 BM biopsies by IHC and compared them with those obtained by CM and FCI. In the CM-IHC comparison, Pearson's r correlation was 0,728 and in the CM-FCI comparison, Pearson's r correlation was 0,782. The correlation between CM-IHC and CM-FCI was slightly higher in this study than in the study by Saft et al. (28); which may be attributable to the difference between case numbers. Their study also found that 17% of patients had higher blast counts by IHC compared to CM, which affected subclassification in MDS. However, in our study 7.4% of the cases were moved to one upper BP group with either IHC or FCI, whereas 13.2% of all cases were classified in one lower BP group. This difference highlights the importance of material adequacy, preparation quality, and interobserver variability in CM.

### Study Limitations

There is a limitation to this study in that only CD34-positive cases were included; however, it is known that not all blasts express CD34. In addition, future studies implementing clinical and survival data will be very useful in determining the most accurate approach in this regard.

## Conclusion

An overall strong correlation was observed between CM, IHC, and FCI in determining the BM blast counts in MDS and AML. In one-fifth of the cases, the BP groups determined by the three methods were different, and this difference led to diagnostic and prognostic classification changes. Therefore, the combination of these three methods will be the most accurate and reliable approach in terms of patient management when determining the BM BP that has diagnostic and prognostic importance.

**Acknowledgements:** The authors extend their sincere thanks to Professor Atilla Özkan from hematology department for his approval of this study and invaluable contributions. The authors also thank the staff of hematology department for their help in preparation of the bone marrow samples.

**Ethics Committee Approval:** Ethical approval for this study was obtained from the Yeditepe University Non-Interventional Clinical

Studies Ethics Committee (decision number: E.83321821-805.02.03-147, date: 10.02.2023). The study protocol conformed to STARD and ethical guidelines established in the World Medical Association Declaration of Helsinki.

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

**Authorship Contributions:** Surgical and Medical Practices - E.H.; Concept - E.H., F.Ö.; Design - E.H., F.Ö.; Data Collection or Processing - E.H.; Analysis or Interpretation - E.H., F.Ö.; Literature Search - E.H.; Writing - E.H.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

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