

ORIGINAL ARTICLE

Proposal of a Diagnostic Workflow for Peripheral Blood Smear Testing Based on Complete Blood Count Flag Analysis

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ABSTRACT

Background: Leukemia requires early detection, especially in acute forms, but standard tests such as bone marrow biopsy and molecular assays are often costly and unavailable in smaller hospitals. In these settings, complete blood count with peripheral blood smear examination provides a practical alternative. Analyzer-generated flags may suggest abnormal cells, yet false positives are common, and without a standardized protocol, smear review is often overlooked, leading to delayed diagnosis. This study aimed to develop and validate a stepwise peripheral blood smear protocol for small hospitals by analyzing blast-flagged complete blood count cases, correlating findings with diagnoses, and facilitating early leukemia detection and timely referral in resource-limited settings.

Methods: This retrospective study was conducted at a small hospital in South Korea. Three datasets were analyzed: 1) 60 patients diagnosed with leukemia (Jan 2020 - Dec 2024), 2) 633 CBC samples flagged with “Blast/Abn Lympho?” or “Left Shift” (Feb 2023 - Jan 2025), and 3) 43,450 CBC samples without any flagging or hematological abnormalities (control group). The flags were automated alerts indicating the presence of immature or abnormal white blood cells (WBCs), prompting PBS review. Descriptive statistics summarized demographic and hematological parameters, and group comparisons were performed using chi-squared and independent *t*-tests, with significance set at $p < 0.05$.

Results: Of the 633 flagged samples, 20 cases (3.2%) showed blast cells on PBS, 19 of which occurred in samples with elevated WBC counts. Several were confirmed as leukemia and referred early for treatment. Flagged cases showed significantly higher WBCs and lower hemoglobin levels than control cases ($p < 0.0001$), supporting the clinical relevance of analyzer flagging.

Conclusions: The proposed workflow facilitates the early detection of hematological malignancies using existing CBC flagging. Our study also underscores the pivotal role of clinical laboratory scientists in diagnostic decision-making, especially in resource-limited environments.

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Supplementary Data

Table S1. Demographic characteristics between flagged and control groups.

Parameter	Flagged group (n = 633)	Control group (n = 43,450)	χ^2 (p-value)
Gender			
Male (%)	55.4%	52.9%	1.430 (0.232)
Female (%)	44.6%	47.1%	
Age (years)			
< 50 (%)	29.9%	38.5%	163.66 (< 0.0001 ***)
50 - 70 (%)	24.1%	37.6%	
> 70 (%)	46.0%	23.9%	

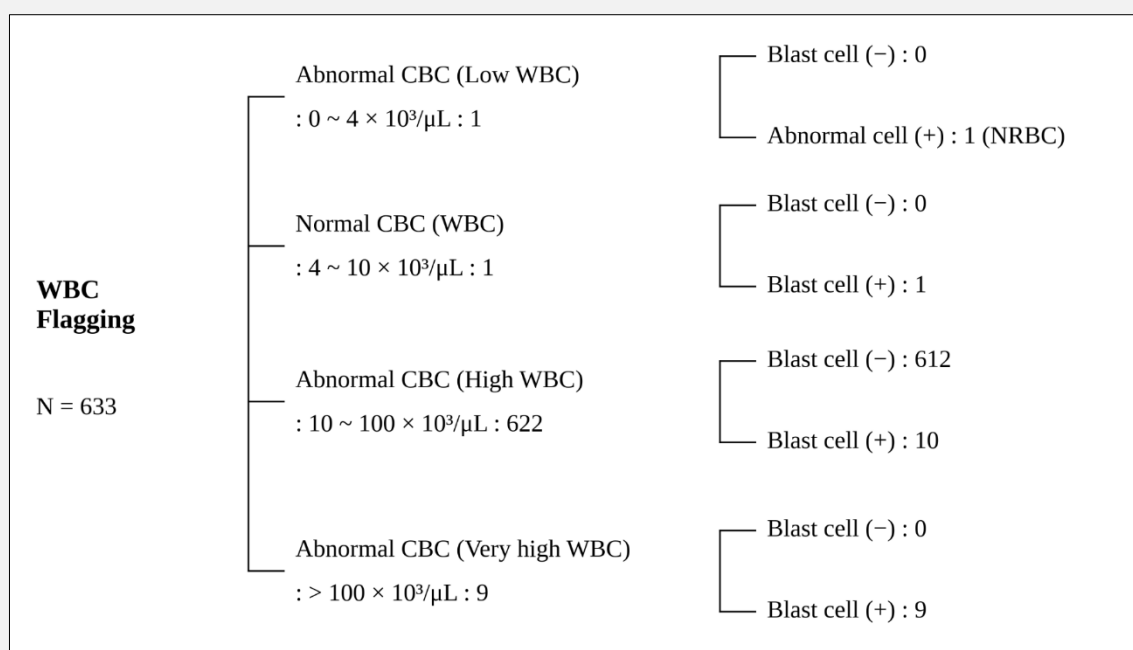
*** - $p < 0.001$.

Figure S1. WBC flagging.

CBC: complete blood count, WBC: white blood cell, NRBC: nucleated red blood cell.

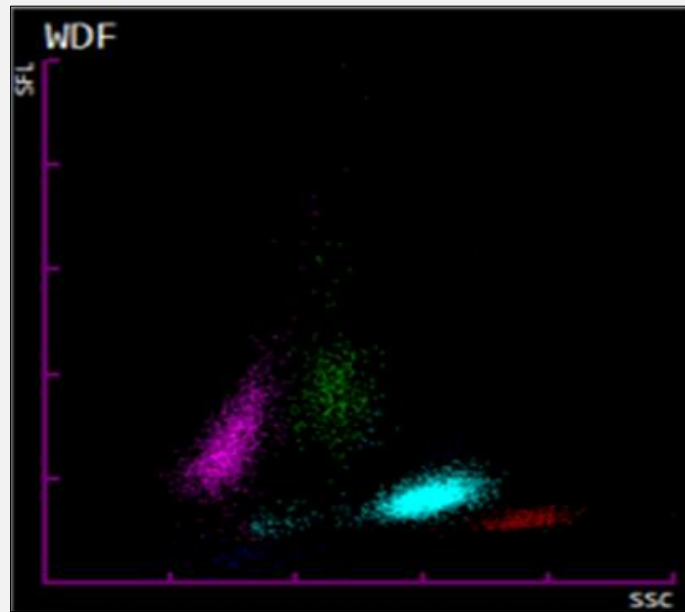


Figure S2. WDF scattergram from a healthy individual.

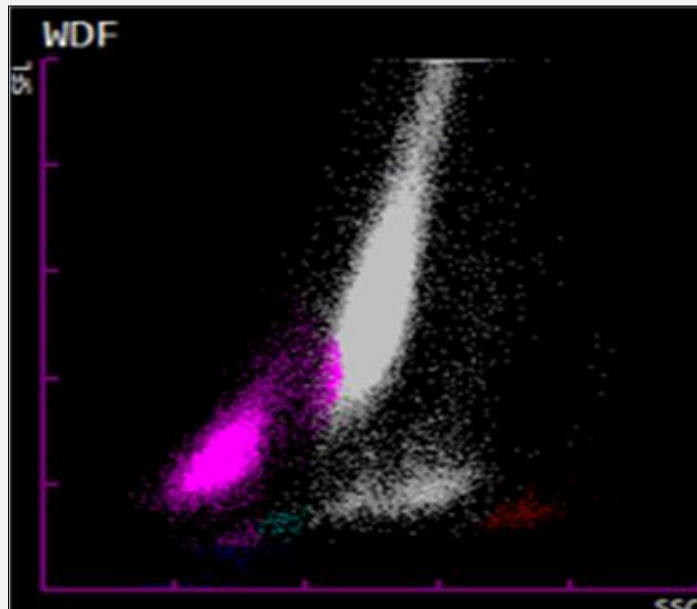


Figure S3. WDF scattergram from a patient with AML.

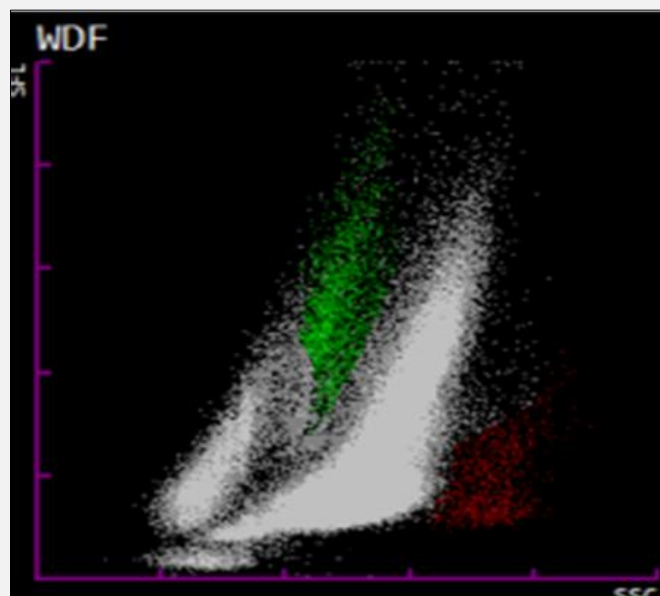


Figure S4. WDF scattergram from a patient with CML.

Figures S2 - S4. Source: Created by the authors from original data generated in this study using the Sysmex XN-Series automated haematology analyser.

Supplementary Appendix

Appendix 1. Standard Operating Procedure (SOP): Blast Flag Detected in CBC - Peripheral Blood Smear Review Protocol.

1. Purpose

This Standard Operating Procedure (SOP) provides a standardized protocol for performing and reporting peripheral blood smear (PBS) tests when blast-related flags are detected in complete blood count (CBC) analyses using automated hematology analyzers in small- and medium-sized hospitals. The aim is to enable early detection of hematologic malignancies - particularly acute leukemia - without the need for expensive equipment, supporting timely referral and appropriate management [1].

2. Scope

Applicable to clinical pathology departments and medical laboratory technologists using automated hematology analyzers such as the Sysmex XN series, particularly in resource-limited settings lacking advanced diagnostic infrastructure, to ensure diagnostic accuracy in hematologic evaluation [2].

3. Definitions & Criteria

Blast-flag criteria: CBC flags such as “Blast/Abn Lympho?”, “Left Shift”, $WBC < 4 \times 10^3/\mu L$ or $> 10 \times 10^3/\mu L$, immature granulocyte (IG) $\geq 2.0\%$ [3].

Blast morphology: Large size, fine chromatin, prominent nucleoli, scant cytoplasm. Auer rods or other leukemic features [2].

4. Responsibilities

Medical Laboratory Technologist: Review CBC results and identify flagged cases; prepare peripheral blood smear slides and perform Wright-Giemsa staining; conduct initial microscopic evaluation at $\times 1,000$ magnification.

Clinical Pathologist (or Attending Physician): Make final interpretations and reporting decisions; determine the need for referral and approve the report.

Laboratory Supervisor: Ensure compliance with the SOP; oversee training and education; manage quality control and equipment maintenance.

5. Materials and Equipment

- Automated hematology analyzer (e.g., Sysmex XN-2000)
- Microscope (including $\times 1,000$ oil immersion lens)
- Slide glass and cover slips
- Wright-Giemsa stain
- Peripheral blood smear preparation kit (including spreader)
- Differential count sheet or Laboratory Information System (LIS)

6. Procedure

Stages	Action Item	Description
Step 1	Review of CBC Results	Proceed with PBS if one or more of the following conditions are met: • Blast/Abn Lympho? flag present • Left Shift flag present • WBC > 10,000/μL or < 4,000/μL • IG (Immature Granulocytes) $\geq$ 2.0% • Unexplained leukocytosis or leukopenia
Step 2	Review of WDF Scattergram	Check for abnormalities in the WDF scattergram: • Expansion of the blast zone • Formation of atypical clusters outside the normal white blood cell distribution
Step 3	Preparation of peripheral blood smear slide	• Apply sample: Place a 1 - 2 μL drop of blood approximately 1 cm from the slide end • Spread smear: Use a spreader slide at a 30° - 45° angle to produce a uniform thin film • Air-dry: Allow the smear to dry completely in air • Fix: Fix the dried smear with methanol • Stain: Stain using Wright-Giemsa according to laboratory protocol • Examine: After staining, air-dry and examine under oil immersion at $\times$ 1,000 magnification
Step 4	Identify abnormal cells	Report if any of the following morphological findings are observed: • Prominent nucleoli • Immature chromatin • Scant cytoplasm • Presence of Auer rods or similar leukemic features
Step 5	Secondary Review and Reporting	In case of suspected blast cells: • Refer the smear to a clinical pathologist for interpretation • If a pathologist is not available, capture high-resolution microscopic images and report directly to the attending physician
Step 6	Clinical referral	Urgent report: "Blast cells suspected - possible leukemia". Recommendation: Refer to hematology-oncology or a tertiary care center.

7. Example Reporting Statement

"Multiple blast-like cells observed on peripheral blood smear. Leukemia is suspected; referral to hematology-oncology is recommended."

8. Quality Control & Training

Daily stain QC using known positive control slides.
Regular competency assessments for technologists.
Documentation of all smear reviews and reports.

9. References:

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2. Palmer L, Briggs C, McFadden S, et al. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. *Int J Lab Hematol* 2015;37(3): 287-303. (PMID: 25728865)
3. Petrone J, Jackups R Jr, Eby CS, et al. Blast flagging of the Sysmex XN-10 hematology analyzer with supervised cell image analysis: impact on quality parameters. *Int J Lab Hematol* 2019; 41(5):601-6. (PMID: 31162809)

10. Revision History

Version	Date	Revisions	Author
1.0	2025.05.06	initial establishment	[Mijin Kang]