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Predictive Value of Automated Hematology Analyzer Flags for Detection of Active Acute Myeloid Leukemia

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ABSTRACT

Objective: Automated hematology analyzer flags may provide early warning signals for hematological malignancies. This study aimed to evaluate the diagnostic performance of Sysmex XN-1000 analyzer flags and to develop a multivariable prediction model for identifying active acute myeloid leukemia (AML) in adult patients.

Methods: A retrospective analysis of 5215 complete blood count (CBC) tests (15 cases and 5200 controls) was conducted at Gazi University Hospital. Data obtained from the hematology analyzer were evaluated. Diagnostic accuracy metrics were calculated for specific Q-flag ("Blasts/Abn Lympho?") and for the interpretive flags of the analyzer. A logistic regression model was employed to identify independent predictors. As a result of these analyses, the best-performing model was selected.

Results: In the multivariable model, "Thrombocytopenia" (odds ratio [OR=57; 95% CI =15-218]) and "Blasts/Abn Lympho?" ≥120 (OR=33; 95% CI =8-135) were independent predictors of active AML. The AML index model demonstrated high diagnostic performance, with a sensitivity of 66.7%, a specificity of 99.8%, a positive predictive value of 56%, a negative predictive value of 99.9%, and an area under the curve (AUC) of 0.894 (95% CI=0.731-0.963) at a cut-off of >0.034. Based on the final multivariable model, an AML index was derived.

Conclusion: The Sysmex XN-1000 flag-based regression model demonstrated high diagnostic accuracy for detection of active AML disease. This method may allow identifying patients who require prompt peripheral blood smear review and further hematological evaluation by allowing for rapid classification of high-risk samples directly from the hematology analyzer. These results, from a retrospective, single-center study with a small sample size, need to be confirmed in larger prospective study designs.

Keywords: Acute, blood cell count, hematologic tests, leukemia, logistic models, myeloid, predictive value of tests, sensitivity and specificity

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INTRODUCTION

A complete blood count (CBC) is a hematological analysis that provides comprehensive information about all types of blood cells in the body.[1] It is a technique used to identify and monitor many different diseases.[2] In the CBC, the main white blood cell (WBC) count, erythrocyte count, platelet count, and leukocyte differential (WBC-Diff) count are determined.[3] White blood cells arise from pluripotent hematopoietic stem cells found in the bone marrow. Once mature, they are released into the peripheral bloodstream.[4]

Within the circulatory system, WBC can be divided into WBC-Diff cells. Differentiated leukocyte cells include lymphocytes, monocytes, neutrophils, eosinophils, and basophils. Any change in any of these cells can affect the quantity of WBC. Therefore, it is important to perform WBC-diff analysis together with WBC.[5]

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In modern automated hematology analyzers, the leukocyte count is obtained by classifying WBC-Diff cells according to their size, volume, and granularity. With current technology, these devices can analyze 5 or 7 types of cells.[6] The automated hematology analyzer used in this study (XN-1000, Sysmex, Kobe, Japan) employs a semiconductor laser and performs analyses through several measurement channels. For WBC-diff, White cell Nucleated (WNR) and White cell Differential (WDF) channels are used. While the absolute number and ratio of basophils and nucleated red blood cells (NRBCs) are measured in the WNR channel, remaining WBC-diff cells (neutrophils, lymphocytes, monocytes, and eosinophils) and immature granulocytes (IG) are analyzed in the WDF channel.[7,8]

The term "flag" in hematology analyzers often refers to signals displayed to operators to identify abnormal cell counts, irregular cell shapes, or other abnormalities that may require further investigation or medical intervention. The presence of abnormal cells on an automated hematology analyzer can provide valuable flags for further evaluation of patients.[9] Consequently, flags serve as a primary screening tool. They are essential for detecting qualitative abnormalities and identifying interferences that may compromise the reliability of quantitative results.[10]

Flags in the WDF channel that may be related to the major hematological malignancies detected on XN-1000 analyzer are shown in the Supplementary Material (Supplementary Table 1). In the study conducted by Becker PH et al[11], high sensitivity was shown in hematological malignancies with the "Blast/Abn Lympho and/or Atypical Lympho" flag analysis in the pediatric age group.

There is also a white precursor cell (WPC) channel that can distinguish between reactive lymphocytes and blasts based on differences in cell membrane composition and DNA labeling density. This contributes to the provision of highly reliable measurement results in clinical practice. Many studies have demonstrated the advantages of using both channels to distinguish between malignant and reactive lymphocytes before blood smear analysis. However, it should be noted that this channel is not present in the XN-1000.[8,9]

In this study, the authors aimed to evaluate the diagnostic performance of Sysmex XN-1000 analyzer flags and to develop a multivariable prediction model for identifying acute myeloid leukemia (AML) in adult patients with a definitive diagnosis.

METHODS

This research was designed as a retrospective study and was conducted between August 2024 and June 2025. The study was approved by the Gazi University Ethics Committee (Approval No: 2025-407; Date: 11.03.2025). Laboratory results were obtained from the hematology analyzer, and the clinical data of the patients were obtained from the hospital information management system (HIMS). The study included 5215 individuals aged 18 and over with a CBC test result obtained in the Hematology Laboratory of Gazi University Hospital during the study period.

Study Population

A total of 306 863 CBC results for adults (≥18 years) were initially identified in the HIMS. From this pool, records were retrieved from 2 main sources: the Hematology Department (n = 10 396) and other departments, including Occupational Health, Ophthalmology, and Check-up units (n = 2991).

Following initial screening, duplicate records and patients meeting the exclusion criteria were removed. In the hematology department, 5127 duplicate results were excluded. Additionally, 2667 patients with hematologic malignancies other than AML and 43 patients with AML in remission were excluded from the study. Among records retrieved from the other departments, 335 records were excluded because of duplication (n = 293), presence of pancreatic malignancy (n = 1), or missing data (n = 41).

The final study population consisted of 5215 patients divided into 2 groups (Figure 1). The case group (n = 15) included 14 patients diagnosed with AML and 1 patient with acute myelofibrosis. The control group (n = 5200) was composed of 2544 patients from the Hematology Department and 2656 patients from other departments (Occupational Health, Ophthalmology, and Check-up units). Definitive diagnostic and referral information was obtained from the HIMS. The system provided definitive diagnoses based on flow cytometry and pathology results.

Selection Flag in XN-1000 Complete Blood Count Analyzer

According to some case studies, flags associated with hematology malignancies are Abn. WBC scattergram, Blasts/Abn Lymph?, Atypical Lympho?, NRBC present, and IG present.[12,13] Based on this information, the patients' CBC results, research parameters, and all flag results were obtained from the hematology analyzers in the Hematology Laboratory of Gazi University Hospital.

Statistical Analysis

Statistical analysis including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), area under the curve (AUC), and regression model analysis was performed using MedCalc's Diagnostics free statistical calculator (MedCalc Software Ltd., Belgium), Statistical Package for the Social Sciences Statistics Version 30 (SPSS Inc., Chicago, IL, USA), JASP for Windows (University of Amsterdam, Netherlands), and XLSTAT statistical software (version 2019.2.2, Addinsoft, France). Continuous variables were compared across independent groups using Mann–Whitney *U*-test. Categorical variables were compared across independent groups using chi-square (χ^2) test or Fisher's exact test. To reduce the small sample bias and increase the stability of the parameter estimates, Firth's penalized likelihood method was used because of the comparatively low number of events (n = 15) relative to the number of predictors, yielding an events-per-variable (EPV) ratio of 7.5.[14] The regression model did not include variables with collinearity (variance inflation factor [VIF] > 5). Logistic regression analyses were performed using XLSTAT statistical software. These evaluations led to the selection of the best suitable model.

RESULTS

Data obtained from the automated hematology analyzer, together with the clinical characteristics of the study population, are presented as continuous and categorical variables in Table 1, and the corresponding

Main Points

- *Acute myeloid leukemia (AML)-specific flag thresholds were defined for the Sysmex XN-1000 analyzer.*
- *An optimal flag pattern was identified for detection of active AML.*
- *The flag-based AML index may provide automated decision support for identifying samples that require urgent peripheral blood smear review.*
- *A possible role during the follow-up of treated AML is hypothetical and requires prospective evaluation.*

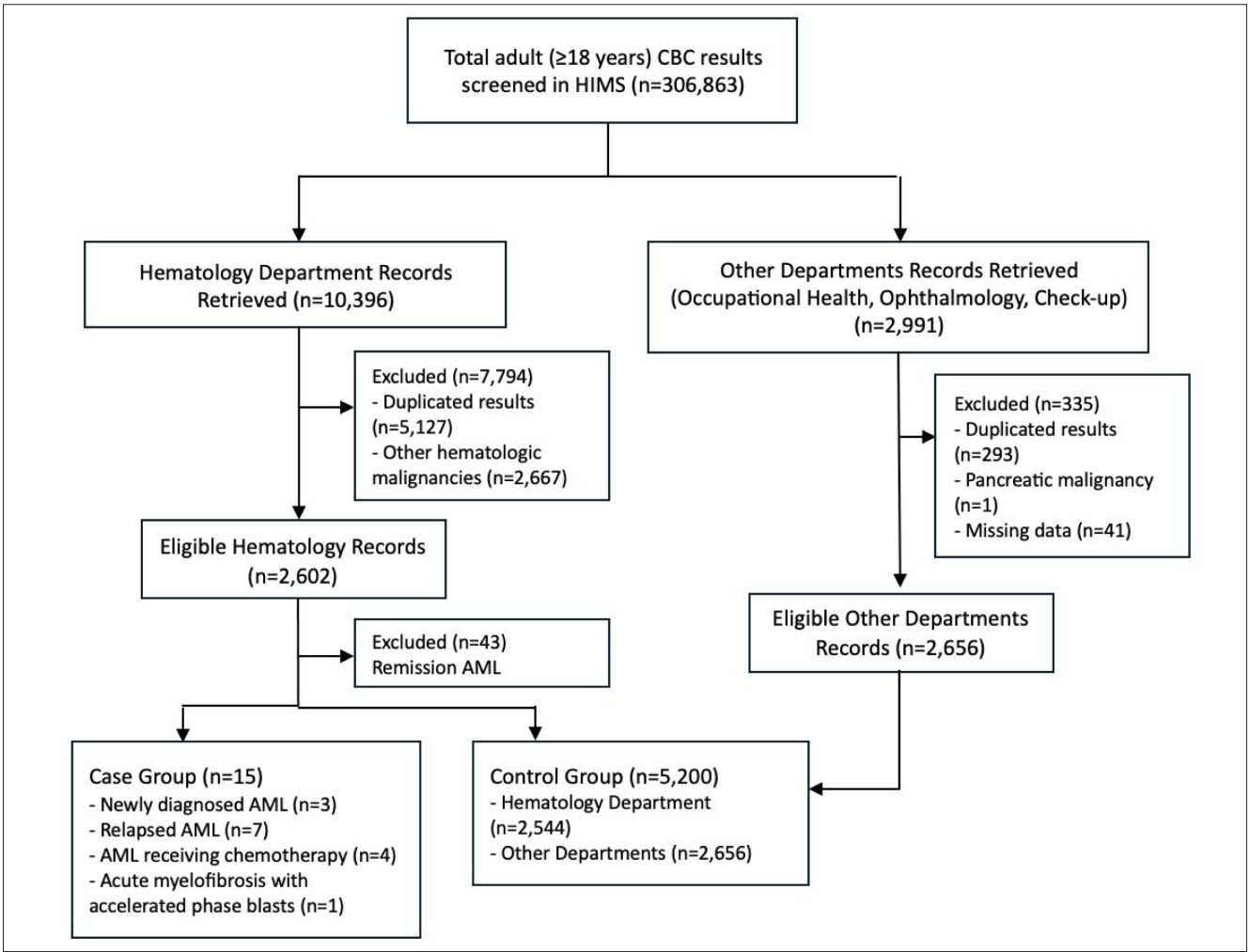

Figure 1. Flowchart of the study population selection process. The diagram illustrates the screening of database records, exclusion criteria (including duplicate results, missing data, non-AML malignancies, and AML in remission), and the final distribution of the case and control groups. AML, acute myeloid leukemia; CBC, complete blood count; HIMS, Hospital Information Management System.

automated analyzer flag data are presented in Table 2. These tables show statistical differences between the control group (n=5200) and the case group (n=15). Patients with AML in remission (n=43) were excluded from both groups, whereas patients with newly diagnosed AML (n=3), acute myelofibrosis with accelerated phase blasts (n=1), relapsed AML (n=7), and non-transplanted patients receiving ongoing chemotherapy for AML (n=4) were included in the case group. Among the 15 cases, the "Leukocytopenia" flag was positive in 9 patients (60%) and "Thrombocytopenia" flag in 10 patients (67%). Subgroup analysis showed that "Leukocytopenia" was predominantly associated with treatment status: of the 9 positive patients, 3 were receiving chemotherapy and 5 had relapsed disease, whereas only 1 was newly diagnosed. "Thrombocytopenia" was positive in 1 patient receiving chemotherapy, 6patients with relapsed disease, and all 3 newly diagnosed patients.

The optimal cut-off for the Q-Flag "Blasts/Abn Lympho?" level was ≥120 (AUC: 0.853; 95% CI: 0.712-0.994). After determining the ≥120, ≥100, and ≥80 Q flag cutoff levels using ROC analysis, they were compared in Table 3. The highest specificity (97%), positive likelihood ratio (LR+) (27.73), and Youden's index (0.771) were observed at the 120-threshold level. The updated "Blasts/Abn Lympho?" subgroup analyses were similar to the "Thrombocytopenia" flag subgroup analyses. Both flags were positive in all 3 newly diagnosed AML.

Univariate and multivariable models were then compared (Table 4). Model 3, which included both "Thrombocytopenia" and "Blasts/Abn Lympho?" (≥120), provided the highest PPV and was therefore used to derive a predictive index for AML (the AML index). Using this formula, ROC analysis identified an optimal cut-off of >0.034. The corresponding values for the AUC (0.894), sensitivity (66.7%), specificity (99.8%), PPV (56%), NPV (99.9%), LR+ (433), and LR– (0.33), and Youden's index (0.665) are shown in Table 5 (Figure 2).

DISCUSSION

This retrospective study evaluated the interpretive flag and Q-Flag parameters of the Sysmex XN-1000 hematology analyzer as first-line laboratory signals for active AML. The principal finding was that combining the "Blasts/Abn Lympho?" and "Thrombocytopenia" flags into a single model (AML index) provided 99.8% specificity and 99.9% NPV, even when the AML prevalence was only 0.3%.

These flags are generated automatically during routine CBC analysis without requiring additional sample volume or additional analytical

Table 1. Clinical and Laboratory Data of Patients with Control Group and Case Group

	Control Group		Case Group			
	n	median [25th-75th]	n	median [25th-75th]	*P*	Rank Biserial Correlation (CI 95%)
Age, years	5200	44 [34-53]	15	62 [43-69]	.005*	0.420 (0.154-0.629)
RBC (10^6/uL)	5200	4.8 [4.5-5.2]	15	2.5 [2.3-2.8]	<.001*	−0.952** (−0.973 to −0.915)
HGB (g/dL)	5200	13.8 [12.8-15.2]	15	8.7 [7.2-9.5]	<.001*	−0.857** (−0.918 to −0.757)
HCT (%)	5200	41.4 [38.8-44.9]	15	25.2 [20.5-28.5]	<.001*	−0.886** (−0.935 to −0.804)
MCV (fL)	5200	87 [84-90]	15	94 [88-100]	<.001*	0.543** (0.308-0.718)
MCH (pg)	5200	29 [28-30]	15	33 [30-34]	<.001*	0.669** (0.475-0.801)
MCHC (g/dL)	5200	33.5 [32.6-34.3]	15	34.6 [33.6-35.2]	.003*	0.445 (0.184-0.648)
PLT (10^3/uL)	5200	266 [227-311]	15	31 [18-88]	<.001*	−0.971**(−0.984 to −0.948)
RDW-SD (fL)	5198	41.5 [39.6-43.8]	15	58.8 [52.2-68.5]	<.001*	0.962** (0.933-0.979)
RDW-CV (%)	5198	13.1 [12.5-13.7]	15	17.5 [15.3-20.7]	<.001*	0.892** (0.814-0.938)
PDW (fL)***	5182	12.1 [10.9-13.5]	14	4 [0-9.8]	<.001*	−0.816** (−0.895 to −0.686)
MPV (fL)***	5182	10.4 [9.8-11.1]	14	4.7 [0-10.1]	.004*	−0.444 (−0.653 to −0.173)
PCT (%)***	5182	0.280 [0.240-0.320]	14	0.005 [0.000-0.085]	<.001*	−0.965** (−0.981 to −0.937)
NRBC# (10^3/uL)	5200	0.000 [0.000-0.000]	15	0.010 [0.000-0.145]	<.001*	0.574** (0.346-0.738)
WBC (10^3/uL)	5200	7.2 [6.0-8.6]	15	2.4 [1.5-21.2]	.026*	−0.333 (−0.564 to −0.053)
NEUT# (10^3/uL)	5200	4.1 [3.2-5.2]	15	1.0 [0.6-11.9]	.027*	−0.331 (−0.563 to −0.051)
LYMPH# (10^3/uL)	5200	2.2 [1.8-2.7]	15	0.9 [0.5-2.3]	.003*	−0.443 (−0.646 to −0.182)
MONO# (10^3/uL)	5200	0.5 [0.4-0.7]	15	0.2 [0.1-1.3]	.124	−0.230 (−0.483 to −0.058)
EO# (10^3/uL)	5200	0.14 [0.08-0.2]	15	0.00 [0.00-0.03]	<.001*	−0.723** (−0.836 to −0.552)
BASO# (10^3/uL)	5200	0.05 [0.03-0.06]	15	0.01 [0.01-0.02]	<.001*	−0.561** (−0.729 to −0.329)
IG# (10^3/uL)	5200	0.02 [0.01-0.03]	15	0.08 [0.00-0.21]	.044*	0.293 (0.009-0.533)
IG%	5200	0.3 [0.2-0.4]	15	4.1 [0.0-7.4]	.024*	0.329 (0.049-0.561)
MicroR (%)***	5199	1.9 [1.2-3.4]	15	2.3 [1.6-5.8]	.188	0.196 (−0.094 to 0.455)
MacroR (%)***	5199	3.9 [3.6-4.2]	15	8.3 [4.3-13.1]	.002*	0.462 (0.205-0.660)
RE-LYMP# (10^3/uL)***	5113	0.06 [0.04-0.09]	15	0.00 [0.00-0.04]	<.001*	−0.578** (−0.740 to −0.351)
NEUT-RI (FI)***	5191	46.5 [44.8-48.2]	15	49.2 [45.3-53.6]	.041*	0.306 (0.024-0.544)
NEUT-GI (SI)***	5191	152.4 [149.7-155.2]	15	151.1 [143.2-153.0]	.083	−0.258 (−0.506 to 0.028)
	n	n (%)	n	n (%)	*P*	OR (CI 95%)
Sex (Female)	5200	3102 (60%)	15	6 (40%)	.186	0.451 (0.132-1.421)

Continuous variables are presented as median [25th - 75th] and were compared between the control and case groups using the Mann–Whitney *U*-test. Categorical variables are presented as n (%) and were compared between the control and case groups using the chi-square (χ^2) test or Fisher's exact test. Unadjusted OR were obtained from univariate logistic regression analyses.

BASO, basophil; EO, eosinophil; HCT, hematocrit; HGB, hemoglobin; IG, immature granulocyte; LYMPH, lymphocyte; MacroR, macrocytic ratio; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MicroR, microcytic ratio; MONO, monocyte; MPV, mean platelet volume; NEUT, neutrophil; NEUT-GI, neutrophil granularity intensity; NEUT-RI, neutrophil reactivity intensity; NRBC, nucleated red blood cell; OR, odds ratio; PCT, plateletcrit; PDW, platelet distribution width; PLT, platelet; RBC, red blood cell; RDW-CV, red cell distribution width–coefficient of variation; RDW-SD, red cell distribution width–standard deviation; RE-LYMP, reactive lymphocytes; WBC, white blood cell.

*Statistical significance level $P < .05$.

**Rank biserial correlation > 0.500: large effect.

***Missing Data.

procedures. This is potentially relevant not only in hematology laboratories but also in emergency departments and intensive care units, where a CBC is frequently requested and the possibility of acute leukemia may not be considered initially.

Flag-based Detection of Active Acute Myeloid Leukemia

In analyzers such as the Sysmex XN series, the probability of the presence of abnormal cells is indicated by a probability index called Q-values (Q-Flags). Q-values range from 0 to 300 and are calculated in increments of 10. The default threshold setting on the analyzer is usually set at 100. When a flag occurs, the Q-value is 100 or higher. The higher the Q-value, the more abnormal the WDF scatterplot is considered. To increase sensitivity and reduce the risk of missing malignant cells, laboratories may choose to lower the Q-value thresholds below the factory setting of 100 (e.g., 80).[10] However, this increases the false positive (FP) rate and leads to unnecessary manual smear reviews. Therefore, new threshold values for Q-Values were established in this study for AML. The Blasts/Abn Lympho? threshold was set higher than the factory setting (120). At this level, sensitivity remained unchanged, and the FP rate decreased. It is important to assess the clinical significance of false negatives (FNs); some studies have shown that even cases of FN missed by flags do not compromise the patient's overall clinical course.[15,16]

One remarkable feature of this study is the availability of a large control group (N = 5200) despite the small number of AML cases (n = 15). This provides a realistic laboratory setup to evaluate flag performance in low prevalence settings. Also, the XN-2000 study by Schoorl et al[17] stressed the significance of examining the performance of the flags in low prevalence settings for clinical validity. It is crucial for the device to prove its discriminating power within this broad normal population as the majority of samples arriving at hematology laboratories are considered normal.

Table 2. Automated Hematology Analyzer Flag Data in the Control and Case Groups

	Control Group		Case Group			
Q-Flag parameters	n	Median [25th-75th]	n	Median [25th-75th]	*P*	Rank Biserial Correlation (CI 95%)
Q-Flag (Blasts/Abn Lympho?)	5200	0 [0-10]	15	150 [120-200]	<.001*	0.706** (0.527-0.825)
Q-Flag (Left Shift?)	5200	0 [0-0]	15	20 [2-110]	<.001*	0.702** (0.522-0.823)
Q-Flag (Atypical Lympho?)	5200	10 [0-20]	15	0 [0-48]	.433	−0.110 (−0.382 to 0.180)
Interpretive flags from the Hematology analyzer (Positive)	n	n (%)	n	n (%)	*P*	OR (CI 95%)
Leukocytopenia	5200	13 (0.3%)	15	9 (60%)	<.001*	562 (179-1766)
Neutropenia	5200	18 (0.3%)	15	6 (40%)	<.001*	192 (63-583)
IG Present	5200	151 (2.9%)	15	6 (40%)	<.001*	23 (8-64)
Anemia	5200	159 (3.1%)	15	12 (80%)	<.001*	113 (34-373)
Thrombocytopenia	5200	24 (0.5%)	15	10 (67%)	<.001*	403 (133-1227)
Blasts/Abn Lympho? (≥100)	5200	237 (4.6%)	15	12 (80%)	<.001*	83 (22-464)
Left Shift?	5200	21 (0.4%)	15	11 (73%)	<.001*	94 (29-309)

Q-Flag values are presented as median [25th–75th] and were compared between the control and case groups using the Mann–Whitney *U*-test. Interpretive flags are presented as n (%) and were compared between the control and case groups using the chi-square (χ^2) test or Fisher's exact test. Unadjusted OR were obtained from univariate logistic regression analyses.

Abn, abnormal; IG, immature granulocyte; Lympho, lymphocyte; OR, odds ratio.

*Statistical significance level: $P < .05$.

**Rank biserial correlation > 0.500: large effect.

The "Blasts/Abn Lympho?" flag, reported from the WDF channel, is one of the most critical flags indicating suspected malignancy.[18] It uses multidimensional signal analysis algorithms to distinguish cell populations with a high nucleus-to-cytoplasm ratio and amorphous granules from other leukocyte subtypes. Therefore, the accuracy of blast flags is determined not only by numerical thresholds but also by the shape, density, and signal scatter patterns of the cell populations.[19] This is consistent with the 80% positivity rate observed in the AML cases and with the ability of the flag to detect even small blast populations: it was positive in the single patient with acute myelofibrosis in accelerated phase. Petrone et al[15] similarly reported that the blast flagging on the XN-10 analyzer offers high sensitivity, which may reduce the need for microscopic confirmation. More sensitive methods, such as manual morphological examination or flow cytometry, are generally still required for a definitive clinical diagnosis of AML.[20] The findings suggest that device-based blast detection may serve as a useful screening tool for identifying patients who require prompt peripheral blood smear review and further hematological evaluation.

The "Left Shift?" flag was positive in 73% of the cases, in keeping with the promyelocyte and myeloblast excess that characterizes acute leukemia. Schoorl et al[17] have noted that the left-shift flag can be particularly elevated in conditions such as reactive neutrophilia and sepsis, and therefore its specificity may vary depending on the context. Therefore, it was not retained in the model due to its lack of specificity.

Cytopenia and Immature Granulocyte Flags as Complementary Signals

An increase in IG is of diagnostic importance in AML. Seghezzi's[21] study reported that an increase in IG parallels the blast load and that the XN series offers high accuracy in detecting IG. As shown in Table 2, the "IG present" flag was associated with active AML (unadjusted OR = 23; 95% CI: 8-64). This finding is clinically significant because the IG ratio is a reliable reflection of "early myeloid proliferation" in peripheral blood.[22] Therefore, IG may be regarded as a supportive flag parameter in the assessment of suspected AML. However, the "IG present" flag showed a lower association with active AML compared to other flags. Therefore, it was not retained in the final model. This lower association may reflect the fact that the IG ratio is more closely related to inflammatory conditions.[23]

All 4 cytopenia-related flags were strongly associated with active AML on univariate analysis, but none was suitable as a stand-alone screening parameter. "Leukocytopenia" showed the highest unadjusted odds ratio (OR[OR = 562; 95% CI: 179-1766]) yet a sensitivity of only 60%.[24] "Anemia" was the most frequent cytopenia in the study's cases, as in the series reported by Chen et al.[24] but carried a lower odds ratio than thrombocytopenia, most plausibly because anemia is common in many chronic diseases and therefore less specific. "Neutropenia" was positive in 6 cases, 5 of whom (83%) had pancytopenia; the patients with isolated neutropenia were all receiving chemotherapy, and the overall pancytopenia rate in the study's cases (33%) was comparable to the 40% reported by Chen et al.[24] Because of its low sensitivity,

Table 3. Diagnostic Performance of Q-Flag Threshold for Blasts/Abn Lympho? Flag

	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR (+)	LR (−)	Youden's Index
Q-Flag (Blasts/Abn Lympho?)	≥120	80	97	7	99.9	27.73	0.21	0.771
Q-Flag (Blasts/Abn Lympho?)	≥100	80	96	5	99.9	20.00	0.21	0.760
Q-Flag (Blasts/Abn Lympho?)	≥80	80	95	4	99.9	14.91	0.21	0.746

Performance metrics were computed using ROC analysis. The optimal cut-off value for the Q-Flag parameter was identified using Youden's J statistic. Sensitivity, specificity, and likelihood ratios (+LR/−LR) were derived from the corresponding contingency tables. AUC values were reported with 95% CIs based on the nonparametric DeLong method.

AUC = 0.853; 95% CI: 0.712-0.994.

AUC, area under the curve; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic.

Table 4. Univariate and Multivariable Logistic Regression Analysis Model

Models	Beta (β)	SE	OR	CI 95%	*P*
Model 1					
Intercept	−7.274	0.535	—	—	—
Blasts/Abn Lympho? (≥120)	4.786	0.611	120	36-397	**<.001**
Model 2					
Intercept	−6.847	0.427	—	—	—
Thrombocytopenia	6.000	0.568	403	133-1227	**<.001**
Model 3					
Intercept	−7.407	0.538	—	—	**<.001**
Thrombocytopenia	4.048	0.682	**57**	15-218	**<.001**
Blasts/Abn Lympho? (≥120)	3.489	0.723	**33**	8-135	**<.001**

AML index formula = 1 / (1 + exp(−(−7.407 + 4.048 * Thrombocytopenia + 3.489 * Blasts/Abn Lympho?))).
OR values in Model 1 and Model 2 represent unadjusted (univariate) estimates from separate logistic regression models; OR values in Model 3 represent adjusted estimates from the multivariable logistic regression model including both predictors.
Abn, abnormal; lympho, lymphocyte; OR, odds ratio.
*Statistical significance level: $P < .001$

"Neutropenia" was also not included in the model. "Thrombocytopenia" (OR = 403; 95% CI: 133-1227) was positive in 67% of cases, reflecting blast infiltration and impaired megakaryopoiesis.[25] But it is also caused by a wide range of non-malignant conditions including viral infection, drug effects, autoimmune mechanisms, sepsis, and hypersplenism.[26] Therefore, thrombocytopenia is not specific for AML, but when evaluated in conjunction with "Blasts/Abn Lympho?" (AML index), it strengthens the positive predictive value of the model.

The Acute Myeloid Leukemia Index

To the authors' knowledge, the AML index is the first attempt to combine XN-1000 flag output into a single probability score for active AML. Its main advantage over the "Blasts/Abn Lympho?" Q-Flag used alone is a substantial gain in specificity and PPV at an acceptable cost in sensitivity: specificity increased from 97% to 99.8% and PPV from 7% to 56%, whereas sensitivity decreased from 80% to 66.7% and the AUC increased from 0.853 to 0.894. This analysis can play a crucial role in the laboratory's decision-making process regarding which samples require urgent smear testing. Furthermore, the high NPV (99.5%) suggests that index-negative samples may be less likely to require urgent microscopic review. However, this finding should be confirmed in prospective studies. It should also be noted that PPV and NPV are prevalence-dependent and can differ significantly in populations where AML prevalence differs.

The complementarity of the 2 components explains this gain. "Blasts/Abn Lympho?" identifies an abnormal cell population,[15] whereas "Thrombocytopenia" reflects marrow failure.[25] They are not specific by themselves. However, they rarely occur simultaneously in a single sample, except in acute leukemia. Adjustment attenuated both ORs substantially (from 120 to 33 for "Blasts/Abn Lympho?" and from 403 to 57 for "Thrombocytopenia"), confirming that the 2 flags share information, yet both remained independently significant ($P < .001$).

Limitations

The study has several limitations that may affect the generalizability of the results. First, the single-center design, the low number of AML cases (n = 15), and the inability to differentiate between AML subtypes limit statistical power. Fifteen events for 2 predictors give an events-per-variable ratio of 7.5, which is below the conventional target of 10.[14] Firth's penalized likelihood was used to reduce small-sample bias, and the final model was restricted to 2 variables selected from candidates that were significant on univariate analysis. However, the risk of overfitting and of optimistically biased performance estimates cannot be excluded, and the wide CIs around the odds ratios reflect this.

Two further limitations should be noted. From a technical perspective, not using the Sysmex XN-20 WPC channel, which can reduce FP rates by distinguishing malignant lymphocytes and blasts according to their cell membrane and DNA content, is a factor that prevents increasing specificity.[27] However, given that the WPC channel is a reflex test, it is not included in routine patient results. In fact, the study incorporates routine test results, and the findings offer insights to inform further analysis of patients. Although the performance approach did not include analyses such as peripheral blood smear

Table 5. Diagnostic Performance of the AML Index

	Cut-off	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR (+)	LR (−)	Youden's Index
AML index	>0.034	0.894	66.7	99.8	56	99.9	433	0.33	0.665
	Control	Case	Total	OR	CI 95%		*P*		
AML index negative	5192	5	5197	—	—		—		
AML index positive	8	10	18	—	—		—		
Total	5200	15	5215	1298	374-4520		<.001		

Diagnostic performance was assessed by ROC analysis of the AML index, and the optimal cut-off was identified using Youden's J statistic. The lower panel presents the 2 × 2 contingency table obtained at this cut-off; the OR was calculated from this table.
AML, acute myeloid leukemia; AUC, area under the curve; CI, confidence interval; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; OR, odds ratio; ROC, receiver operating characteristic.

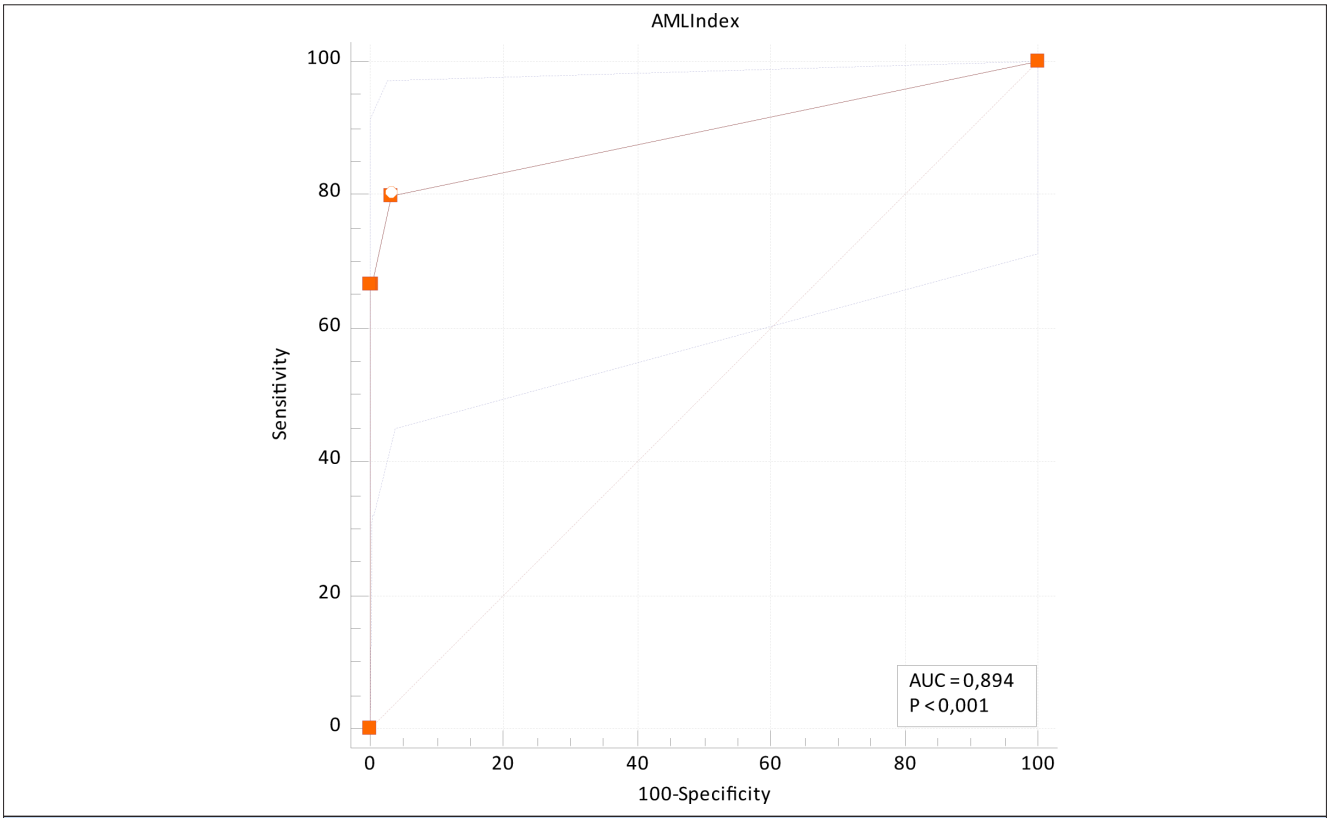

Figure 2. Receiver operating characteristic (ROC) curve of the AML index (AUC = 0.894; optimal cut-off >0.034). AML, acute myeloid leukemia; AUC, area under the curve.

and flow cytometry, the authors were able to access complete patient information from the HIMS. The analyses were performed according to definitive diagnoses. Finally, because PPV and NPV depend on disease prevalence, the predictive values reported here apply to a setting with a very low AML prevalence (0.3%). They may differ substantially in populations with a different prevalence of AML.

CONCLUSION

This study suggests that the Q-flag parameters of the Sysmex XN-1000 hematology analyzer, particularly the "Blasts/Abn Lymph?" flag, may be useful for the identification of patients with suspected AML. The "Thrombocytopenia" flag appeared to contribute complementary diagnostic information when combined with the "Blasts/Abn Lymph?" flagging. Given the retrospective design and limited number of AML cases, these findings should be considered hypothesis-generating. If validated in larger, prospective, multicenter studies, automated flag-based AML detection algorithms can support more timely diagnostic pathways. Further prospective studies are needed to refine flag-based algorithms, assess their cost-effectiveness, and evaluate their impact on clinical outcomes in AML.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethical committee approval was received from the Gazi University Ethics Committee (Approval No: 2025-407; Date: 03.03.2025).

Informed Consent: Informed consent was not necessary due to the retrospective nature of the study.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Flags of Hematology Analyzers relevant to hematology malignancies	
Flags	**Meaning**
Abn. WBC Scattergram	Abnormal WBC Scattergram
Leukocytopenia	Low leukocyte count
Neutropenia	Low neutrophil count
IG present	Increased immature granulocyte
Anemia	Anemia presence
Thrombocytopenia	Low platelet count
Blasts/Abn Lympho?	Possibility that blasts are present/ Possibility of abnormal lymphocytes
Left Shift?	Possibility of left shift
Atypical lympho?	Possibility of atypical lymphocytes
RBC Agglutination?	Possibility of RBC Agglutination