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Machine Learning Models Improve the Diagnostic Yield of Peripheral Blood Flow Cytometry

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Machine Learning Models Improve the Diagnostic Yield of Peripheral Blood Flow Cytometry

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ABSTRACT

Objectives: Peripheral blood flow cytometry (PBFC) is useful for evaluating circulating hematologic malignancies (HM) but has limited diagnostic value for screening. We used machine learning to evaluate whether clinical history and CBC/differential parameters could improve PBFC utilization.

Methods: PBFC cases with concurrent/recent CBC/differential were split into training ($n = 626$) and test ($n = 159$) cohorts. We classified PBFC results with abnormal blast/lymphoid populations as positive and used two models to predict results.

Results: Positive PBFC results were seen in 58% and 21% of training cases with and without prior HM ($P < .001$). % neutrophils, absolute lymphocyte count, and % blasts/other cells differed significantly between positive and negative PBFC groups (areas under the curve [AUC] > 0.7). Among test cases, a decision tree model achieved 98% sensitivity and 65% specificity (AUC = 0.906). A logistic regression model achieved 100% sensitivity and 54% specificity (AUC = 0.919).

Conclusions: We outline machine learning-based triaging strategies to decrease unnecessary utilization of PBFC by 35% to 40%.

Flow cytometry is a rapid and sensitive diagnostic test and remains an essential component of the workup for hematologic malignancies (HM).¹⁻³ Peripheral blood flow cytometry (PBFC) analysis is increasingly used for evaluating and monitoring circulating HM, but is often negative when used as a screening test in patients for whom there is low clinical suspicion for the presence of new HM.^{4,5} The 2006 Bethesda International Consensus group recommended that PBFC be performed for the following clinical indications: cytopenias other than isolated anemia, leukocytosis (including lymphocytosis, monocytosis, and eosinophilia), or the presence of atypical cells or blasts in the peripheral blood.^{6,7} In addition, the consensus group agreed that flow cytometry is indicated for monitoring patients with a previously diagnosed HM, as determination of relapse, progression, and minimal residual disease impacts clinical management.

However, both published evidence⁸ and our own experience suggest that even when ordered using the current consensus indications, many PBFC workups produce no significant findings and provide little to no diagnostic value. Considering the limited diagnostic value of PBFC results in some patients and the pressure hospitals, health systems, and laboratories are under to control costs and ensure judicious use of laboratory resources, we questioned whether current approaches to ordering PBFC represent optimal utilization.

Kovach et al⁹ previously addressed a similar question regarding cerebral spinal fluid (CSF) flow cytometry and demonstrated that a triaging algorithm using history of

HM and CSF total nucleated cell (TNC) counts in combination with morphology on cytology improved the diagnostic value of CSF flow cytometry without the loss of clinically important data. Specifically, they found that flow cytometry testing on CSF samples with low cellularity and from patients without a history of HM were of limited clinical utility. Our institution implemented a CSF specimen deferral policy based on these criteria starting in 2016, whereby specimens are accepted if the patient has a history of HM or a TNC count of more than 5 cells/ μ L. Specimens not meeting these criteria are deferred; clinicians are notified regarding deferred specimens and are asked to speak with the laboratory if they feel the testing is indicated despite not meeting these criteria.

However, the triaging algorithm used for CSF flow cytometry is not directly applicable to PBFC and the development of a similar triaging strategy for PBFC utilization poses several additional challenges. First, the laboratory receives many more PBFC orders and for more diverse indications than CSF flow cytometry, at least in part due to ease of specimen procurement (routine blood draw vs lumbar puncture). Second, HMs involving the peripheral blood are much more prevalent than those involving the central nervous system. Third and most significantly, not only does peripheral blood generally have much higher cell counts, thus rendering cellularity criteria similar to those used for CSF meaningless, but also the many parameters associated with complete blood counts with differentials (CBC/diff) demonstrate substantial variance within a healthy population as compared to normal variation in CSF cell counts. To overcome these challenges, in this study, we used machine learning to develop practical models using clinical history of HM and CBC/diff parameters to predict PBFC outcomes with the goal of optimizing PBFC utilization.

Materials and Methods

As described in greater detail below, we applied machine learning to predict whether a PBFC test will generate significant diagnostic findings using routine hematologic laboratory data and clinical history of HM typically available prior to PBFC testing.

Study Population

The study was approved by the Massachusetts General Hospital (MGH)/Partners HealthCare Institutional Review Board. We included all PBFC cases performed in our hospital's flow cytometry laboratory in 2017 that were associated with a concurrent or recent

(within the prior 6 weeks) CBC/diff order. Because our emphasis was on the development of an algorithm optimized for prospective PBFC triaging, we considered only CBC/diff results reported concurrent with or prior to the PBFC testing. While, per reflex protocol, the laboratory would usually add a CBC/diff onto the PBFC if one was not ordered upfront, add-on CBC/diff results reported after the PBFC were excluded based on these inclusion criteria and intended model application. A total of 854 PBFC cases were identified, with 41 (5%) excluded due to lack of a concurrent or recent CBC and an additional 29 (3%) excluded because the associated CBCs were without differentials. Thus, the final study cohort was composed of 784 PBFC samples (from 744 unique patients) with a concurrent or recent CBC/diff order.

Predictors

Basic demographic information and clinical data, including prior history of HM (any lymphoid or myeloid neoplasm, including monoclonal gammopathy and monoclonal B lymphocytosis), were collected from patient records through review of clinical notes. The CBC/diff parameters analyzed included: WBC, hemoglobin, hematocrit, platelet count, % lymphocytes, absolute lymphocyte count (ALC), % neutrophils, absolute neutrophil count, % monocytes, absolute monocyte count, % other cells, and % blasts. In our hematology laboratory, the term "other cells" is used to denote the presence of cells that the technologist cannot classify with certainty based on morphology. In many cases, these cells are confirmed to be blasts after further diagnostic workup (eg, PBFC, bone marrow evaluation). Therefore, % other cells and % blasts were combined into a single parameter, % blasts/other cells, in our modeling analysis.

Flow Cytometry Performance and Interpretation

All peripheral blood samples were received and processed by the MGH Clinical Flow Cytometry Laboratory. All specimens were processed within 24 hours of specimen collection, with the rare exception of samples collected over a weekend, which were placed in Streck Cell Preservative. Samples were processed using methods, reagents, and instrumentation described previously.⁹ In this study, a positive PBFC result was defined as one with an abnormal blast or lymphoid population (B or T cell) as determined by the hematopathologist at the time of original evaluation. A negative PBFC result was defined as one with no abnormal findings or with findings of limited clinical significance, including low-count monoclonal B-cell lymphocytosis (absolute cell count $<500/\mu$ L) and small circulating myeloid blast populations consistent with

bone marrow recovery after treatment in patients with a history of HM, as determined by the hematopathologist at the time of original evaluation. Flow cytometry results were extracted based on manual report review.

Univariate Analysis

Statistical analyses and modeling were performed using the open-source R statistical programming language (version 3.4.1, available at <https://www.r-project.org/>). χ^2 tests were used to compare categorical variables and Mann-Whitney *U* tests with areas under the curve (AUCs) were used to compare continuous variables (ie, CBC/diff parameters). χ^2 and Mann-Whitney *U* tests were performed as implemented in the R base stats package, and univariate AUCs were derived from the Mann-Whitney *U* test statistic using standard formulas.

Multivariate Prediction Analysis

The PBFC samples were randomly split 80%/20% into training and test cohorts, respectively. All models were trained on the training cohort and their performance was evaluated on the test cohort. A recursive partitioning algorithm (rpart package) was used to generate a decision tree; no optional control parameters were specified for the rpart function. The generalized linear model (glm) function including the R base stats package with binomial family type was used to generate a logistic regression model to evaluate the predictors of PBFC results (binary outcome). For each model, optimal prediction thresholds for distinguishing positive from negative predictions were selected empirically based on training data results; thresholds were chosen to maximize high sensitivity (abnormal PBFC correctly identified) at the expense of specificity to minimize the risk of deferring potentially useful PBFC testing, as that was deemed to be the most clinically acceptable approach. Confidence intervals around sensitivity and specificity were calculated using the binom package. Receiver operating characteristic (ROC) curves were generated for each model using the pROC package. *P* values of $< .05$ were considered statistically significant.

Results

Demographic Features of the Training and Test Cohorts

The training cohort ($n = 626$) and the test cohort ($n = 159$) had mean ages of 60.1 years (standard deviation, 18.9 years) and 59.6 years (standard deviation, 18.1 years), respectively ($P = .778$); 289 (46%), and 56 (35%) of the training and test cohorts, respectively, were female ($P = .016$); 230 (37%) and 54 (34%) of the

training and test cohorts, respectively, had a history of HM ($P = .567$).

Correlation of Clinical and Hematologic Predictors With PBFC Results in the Training Cohort

In the training cohort, 246 cases (39%) were classified as having a positive PBFC result. Within these positive results, 135 (55%) had an abnormal lymphoid population and 111 (45%) had an abnormal blast population. Within the 380 cases (61%) classified as having a negative PBFC result, 328 (86%) had no abnormal populations and 52 (14%) had findings of limited clinical significance.

Positive PBFC results were seen in 143/246 (58%) and 81/380 (21%) of cases with and without a history of HM, respectively ($P < .001$). Thus, patients with a history of HM accounted for a significantly higher proportion of positive vs negative PBFC cases (58% vs 21%, $P < .001$) (Figure 1). For the CBC/diff parameters analyzed, the overall distribution of each parameter overlapped between the positive and negative PBFC groups, although some distributions were skewed in one direction for positive PBFC cases (Figure 2). However, the parameters that showed the most significant difference between the positive and negative PBFC groups (indicated by an AUC ≥ 0.7)¹⁰ were % neutrophils, ALC, and % blasts/other cells (Table 1). Thus, these parameters were chosen as predictors for further modeling.

Predictive Models of PBFC Result in the Test Cohort

The following parameters were used to train the models: % neutrophils, ALC, % blasts/other cells (dichotomized: 1 if $>0\%$ and 0 if 0%), and history of HM (dichotomized: 1 = TRUE and 0 = FALSE). A single decision

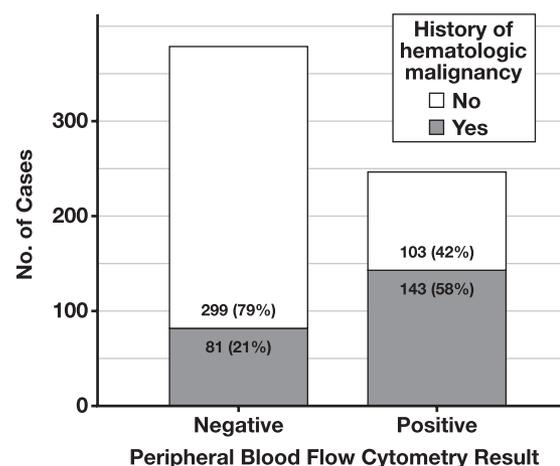


Figure 1 Peripheral blood flow cytometry results in the training cohort stratified by history of hematologic malignancy.

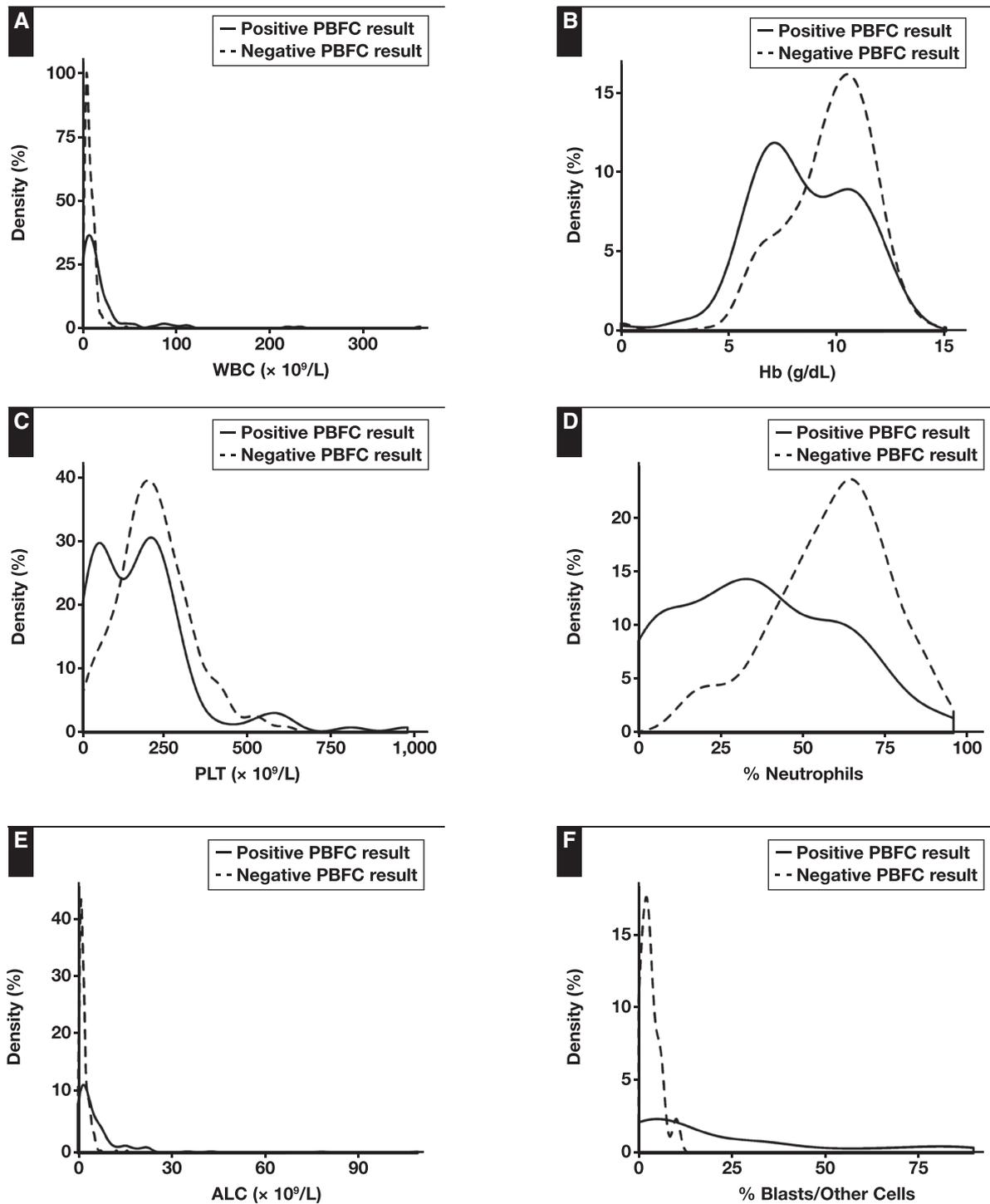


Figure 2 Density plots demonstrating distributions of (A) white blood cell count (WBC), (B) hemoglobin (Hb), (C) platelet count (PLT), (D) % neutrophils, (E) absolute lymphocyte count (ALC), and (F) % blasts/other cells between the positive and negative peripheral blood flow cytometry (PBFC) groups.

tree model was initially selected to facilitate generation of a practical clinical algorithm. The decision tree was trained on the training cohort and an optimal cutoff was selected to maximize sensitivity. Specifically, the relevant decision nodes were % neutrophils, % blasts/other cells, and history

of HM; all PBFC cases with % neutrophils equal to or greater than 41.5%, no blasts or other cells, and no history of HM were accurately classified as negative **Figure 3**.

On the test cohort, the single decision tree model achieved a sensitivity of 98% and specificity of 65% **Table 2**

Table 1**Differences in CBC With Differential Parameters Between Positive vs Negative PBFC Groups in the Training Cohort**

CBC/diff Parameter	Mean of Positive PBFC Group	Mean of Negative PBFC Group	<i>P</i> ^a	AUC ^b
WBC, × 10 ⁹ /L	24.5	8.5	<.001	0.625
HCT, %	32.6	35.5	<.001	0.601
Hb, g/dL	10.8	11.9	<.001	0.612
PLT, × 10 ⁹ /L	145.2	204.4	<.001	0.679
% Neutrophils	33.9	58.9	<.001	0.800
ANC, × 10 ⁹ cells/L	7.2	5.5	.020	0.555
% Lymphocytes	42.7	27.0	<.001	0.676
ALC, × 10 ⁹ cells/L	11.2	1.9	<.001	0.711
% Monocytes	7.4	9.1	<.001	0.638
AMC, × 10 ⁹ cells/L	1.1	0.9	.464	0.518
% Blasts/other cells	24.9	4.2	<.001	0.829

ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; AUC, area under the curve; CBC, complete blood cell count; diff, differentials; Hb, hemoglobin; HCT, hematocrit; PBFC, peripheral blood flow cytometry; PLT, platelet count; WBC, white blood cell count.

^aMann-Whitney *U* test. Bold values indicate significant *P* values.

^bBold values indicate fair (0.7-0.8) to good (0.8-0.9) AUCs.

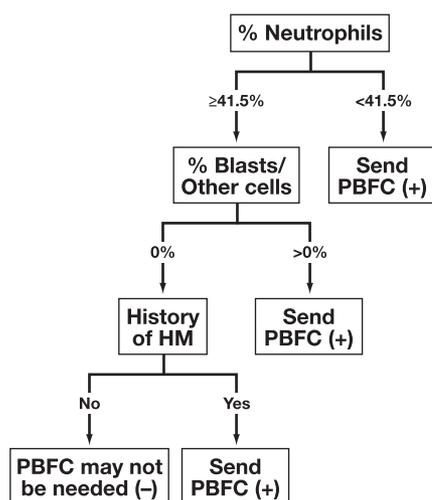


Figure 3 Proposed decision tree model for triaging peripheral blood flow cytometry specimens. HM, hematologic malignancy; PBFC, peripheral blood flow cytometry.

Table 2**Performance of Decision Tree Algorithm in the Test Cohort**

Decision Tree Predicted Result	PBFC Result		Total
	Positive	Negative	
Positive	55	36	91
Negative	1	67	68
Total	56	103	159

PBFC, peripheral blood flow cytometry.

and **Table 3**, with an AUC of 0.906 **Figure 4A**. For comparison, a logistic regression model was created and demonstrated a sensitivity of 100% and specificity of 54% with an AUC of 0.919 **Table 4** and **Figure 4B**; % neutrophils, ALC, the presence of blasts/other cells, and history of HM all emerged as significant predictors **Table 5**.

False-positive rates for the decision tree and logistic regression models were 35% and 46%, respectively, while

false-negative rates were 2% and 0%, respectively **Table 3**. Of note, the only case classified as a false negative in the decision tree model was that of an asymptomatic patient with intermittent mild leukocytosis and normal differential for over 15 years and no other significant past medical history; the patient's PBFC included in the study showed 0.1% blasts (classified as a positive result in our study based on identification of an abnormal blast population in a patient with no history of HM), but a follow-up PBFC was negative.

Discussion

Our study proposes a decision tree model for triaging PBFC specimens based on history of HM and CBC/diff parameters. Specifically, all patients with % neutrophils equal to or greater than 41.5%, no blasts or other cells, and no history of HM had negative PBFC results in our cohort. Additionally, a logistic regression model found that lower % neutrophils, higher ALC, the presence of any blasts or other cells, and a history of HM were significant predictors of a positive PBFC result. Both models had a practical sensitivity of 100%, as the only case classified as a false negative in the decision tree model did not reveal clinical evidence of disease, and a follow-up PBFC was negative. Given false-positive rates (1-specificity) of 35% with the decision tree model and 46% with the logistic regression model **Table 5**, these triaging strategies could potentially defer 35% to 40% of all PBFC (with concurrent or recent CBC/diff). The deferred tests would be expected to produce no diagnostic findings, and thus the triaging protocol would not be expected to miss any clinically significant outcomes.

As expected, a significantly higher proportion of patients with a history of HM had positive PBFC results,

Table 3**Performance Measures of the Decision Tree and Logistic Regression Models in the Test Cohort**

Model	Sensitivity, % (95% CI)	Specificity, % (95% CI)	False-Positive Rate, % (95% CI)	False-Negative Rate, % (95% CI)
Decision tree	98.2 (90.6-99.7)	65.0 (55.5-73.6)	35.0 (26.4-44.5)	1.8 (0.3-9.4)
Logistic regression	100 (93.6-100)	54.4 (44.8-63.7)	45.6 (36.3-55.2)	0 (0-6.4)

CI, confidence interval.

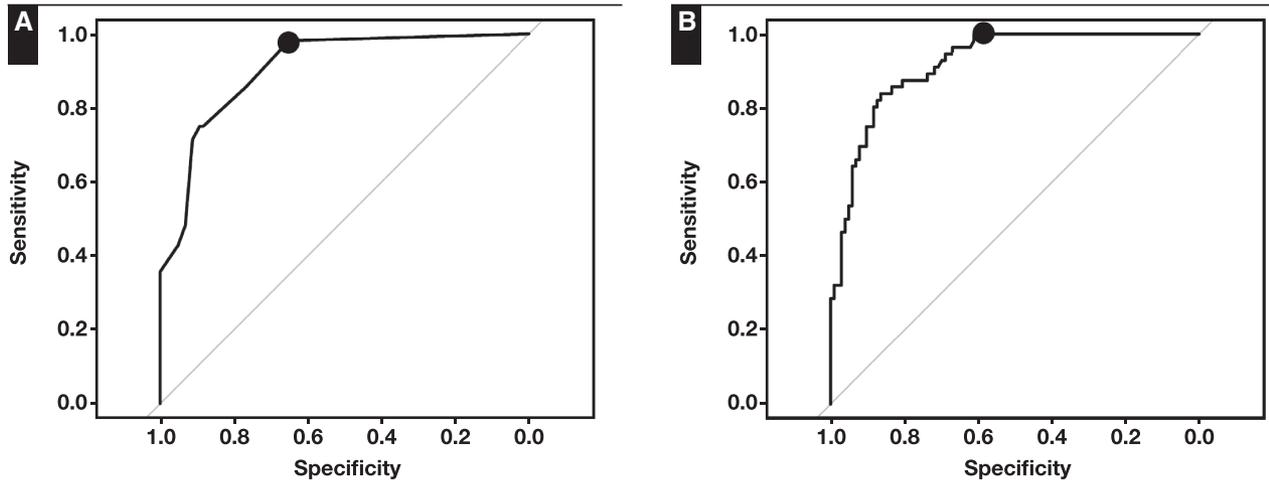


Figure 4 Receiver operating characteristic curves for the (A) decision tree and (B) logistic regression models. Note the decision cutoffs (black dots) chosen to maximize sensitivity and minimize false-negative predictions. **A**, Sensitivity = 98%; specificity = 65%; area under the curve (AUC) = 0.906. **B**, Sensitivity = 100%; specificity = 54%; AUC = 0.919.

Table 4**Performance of Logistic Regression Model in the Test Cohort**

Logistic Regression Predicted Result	PBFC Result		Total
	Positive	Negative	
Positive	56	47	103
Negative	0	56	56
Total	56	103	159

PBFC, peripheral blood flow cytometry.

Table 5**Logistic Regression Model With Normalized Predictors**

Predictor	OR	95% CI	P
% Neutrophils	0.67	0.52-0.87	.003
ALC, $\times 10^9$ cells/L	1.33	1.21-1.47	<.001
Presence of blasts/other cells	2.24	1.80-2.82	<.001
History of HM	5.00	3.25-7.76	<.001

ALC, absolute lymphocyte count; CI, confidence interval; HM, hematologic malignancy; OR, odds ratio.

and both models independently identified history of HM as a significant predictor of a positive test result. This finding is consistent with clinical intuition and consensus recommendations that PBFC is indicated for monitoring all patients with a history of HM. Because pathologist/hematopathologist review of the peripheral blood smear and morphologic identification of atypical cells/blasts is

an important part of the patient's workup and is cited as an indication for performing PBFC testing, we included manual identification of blasts or other cells by hematology technicians, reported as part of the CBC/diff, in our models. The presence of any number of blasts or other cells from the CBC/diff proved to be significant predictors of positive PBFC, supporting the practice that morphologic review of the peripheral blood smear is an important adjunct to PBFC testing.

Previous studies have proposed algorithms for triaging PBFC and have found that increasing patient age and ALC were key predictors for a positive PBFC result; however, these studies focused on older patients and only evaluated the detection of chronic B-cell lymphoproliferative disorders (B-LPDs).^{4,11-15} To summarize their findings, patients older than 50 to 55 years old and ALC higher than 5.0×10^9 cells/L were found to be independent predictors of B-LPDs. Based on these criteria, Oberley et al^{4,12} developed a diagnostic algorithm where patients who fulfilled both criteria received a full B-LPD flow cytometry panel, while those with a low pretest probability of B-LPD received a limited screening flow cytometry panel which, if positive, reflexed to the full flow cytometry panel (reflex rate of approximately 20%). Identification of atypical lymphocytes on peripheral blood smear was found to further improve the diagnostic yield.⁸ We also evaluated the

ALC in our models; the ALC was significantly higher in the positive PBFC group with a fair AUC of 0.711.¹⁰ This parameter was not found to be a node in the decision tree model though it was a significant predictor in the logistic regression model.

Healey et al¹⁵ proposed the first decision tree analysis model for triaging PBFC by identifying significant predictors from clinical information and laboratory test results using logistic regression. As with other studies, the model was only applicable for predicting B-LPDs. In addition to a subset of CBC/diff parameters, they also evaluated numerous other serum laboratory values; the resulting decision tree showed that PBFC is mostly noninformative in patients with an ALC less than 5.0×10^9 cells/L, age younger than 50 years, and age 50 years or older with ferritin 450 $\mu\text{g/L}$ or higher. In their cohort, their decision tree correctly identified 26% of negative PBFC cases. Our models improve upon previous methods by including all PBFC cases sent for any indication, including for the evaluation of acute leukemias and myeloid neoplasms (ie, not limited to B-LPDs). Furthermore, our decision tree operates on minimal clinical and CBC/diff data without the need for additional laboratory results and accurately predicted 41% of negative PBFC cases. Although it would not have been unreasonable for us to include patient age as a possible predictor in our model, particularly given its significance in Healey et al,¹⁵ our goal was to develop a model that relied primarily on laboratory data and history of HM as recommended by 2006 Bethesda International Consensus Group.^{6,7} Given that we used only a moderate number of cases for model training, it was important to select potential predictors judiciously to reduce the risk of overfitting.

We plan to implement this algorithm or a minor variation of it at our hospital to improve PBFC test utilization. We have developed a preliminary clinical decision support alert based on the algorithm for use within our electronic health record system that has been running in “silent mode” to collect data, which we plan to use for prospective validation and optimization of the alert. Assuming successful validation, we intend to implement the decision support to alert clinicians to PBFC orders that are unlikely to be of high value. This would also allow us to offer a reflex protocol, CBC/diff with reflex to PBFC, in instances when a PBFC order is placed in the absence of recent CBC/diff data. Review of medical records in cases excluded from our analysis for this reason indicates that most PBFC orders were to evaluate for the possibility of an underlying lymphoproliferative disorder in the setting of autoimmune or rheumatologic disease or immune deficiency. Less common reasons included evaluation for a CBC/diff abnormality identified more

than 6 months prior, workup of lymphadenopathy, other organ enlargement or mass on imaging, or evaluation of systemic symptoms such as fever, weight loss, or rash. The same reasons for ordering PBFC were seen among cases included in the analysis with a negative decision tree prediction (ie, true-negative cases), but in this group most PBFC orders were to evaluate for CBC/diff abnormalities that did not meet decision tree cutoffs. Offering CBC/diff with reflex PBFC in the context of a validated clinical decision support tool could assure clinicians that further evaluation will be performed in cases that warrant it without the patient having to return for an additional blood draw.

The clinical decision support alert and reflex protocol described above may help to ensure more appropriate use of laboratory resources. Assuming a roughly approximated reimbursement rate of \$800, implementing this protocol at just our hospital could be expected to save about \$250,000/year based on a specificity of approximately 65% ($\$250,000 = \text{approximately } 800 \text{ tests per year with concurrent CBC/diff} \times \text{approximately } 60\% \text{ negative rate} \times \text{approximately } 65\% \text{ specificity} \times \$800/\text{test}$). Although the variable cost to the laboratory of performing this testing may be less than the typical reimbursement rate, our estimated savings provide some reflection of the cost to payors and patients in an era where laboratories and health care institutions face increasing pressure to control costs and ensure judicious use of resources.

Despite the potential value of our algorithm in triaging PBFC orders, the algorithm would likely not be appropriate for use in screening a general population. We developed and validated the algorithm only in patients for whom a clinician had ordered PBFC, as this group of patients would almost certainly have a much higher pretest likelihood of HM than the general population. For additional perspective, we applied our algorithm against all outpatient CBC/diff tests performed by our laboratory or certain affiliated locations over a 6-month period. If the triaging algorithm had been used to screen this general population, it would have suggested that approximately 8% (approximately 7,200 tests) of the nearly 90,000 CBC/diff tests were in patients who could benefit from flow cytometry. (This number does not include any additional patients with a history of HM whose samples would have been flagged based on that criterion alone.) This was primarily due to patients with neutrophil percentages less than 41.5%. Given that other mechanisms exist to identify patients who would benefit from PBFC based on CBC/diff findings (eg, first-time blasts or other cells on manual differential prompting hematopathologist review), this appears to be an inappropriate use of our algorithm.

Our study is subject to several limitations. Our cohort is from a single academic tertiary-care level institution with patient populations, clinician ordering practices, and specific flow cytometry analysis protocols that may differ from other institutions and facilities. We would not consider this protocol suitable for implementation at other institutions without an appropriate and ideally prospective validation at each respective institution considering implementation. Indeed, as noted above, we plan for additional prospective validation even at our own institution prior to full clinical implementation. Another consideration is that our protocol requires CBC/diff test results, which were not ordered in 70 (8%) of the PBFC cases originally identified. In practice, this raises the issue that our models are not applicable in patients for whom only PBFC—but no CBC/diff—is ordered. Perhaps of greater concern is that patients are being screened for a possible diagnosis of HM without accurate, up-to-date peripheral blood counts, which provide the main indications for PBFC testing. One recently implemented improvement to our test ordering system that may largely address this issue is the creation of an order panel for PBFC that automatically includes a CBC/diff, and future implementation of the clinical decision support alert and reflex protocol described above would help address this issue further.

Overall, our results demonstrate the power of utilizing machine learning that incorporates readily available clinical and laboratory parameters to create improved models for the accurate triaging of PBFC testing, thereby dramatically decreasing unnecessary utilization and improving the diagnostic yield of these laboratory tests.

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QUESTIONNAIRE

H2 (21)

Machine Learning Models Improve the Diagnostic Yield of Peripheral Blood Flow Cytometry

INSTRUCTIONS

- Read through the article and answer the multiple-choice questions provided below.
- **Some questions may have more than one correct answer;** in which case you must mark **all** the correct answers.

Question 1: The 2006 Bethesda International Consensus group recommended that peripheral bloodflow cytometry (PBFC) be performed for which of the following clinical indications?

- A:** Cytopenias other than isolated anemia
- B:** Leukocytosis
- C:** The presence of atypical cells or blasts in the peripheral blood
- D:** None of the above

Question 2: Is it **TRUE** that when *Kovach et al.* questioned whether current approaches to ordering PBFC represented optimal utilization of cerebral spinal fluid (CSF) flow cytometry, they demonstrated that a triaging algorithm using history of hematologic malignancy (HM) and CSF total nucleated cell (TNC) counts in combination with morphology on cytology, improved the diagnostic value of CSF flow cytometry without the loss of clinically important data?

- A:** YES
- B:** NO

Question 3: The development of a similar triaging strategy for PBFC utilization poses which of the following challenges?

- A:** The laboratory receives many more PBFC orders and for more diverse indications than CSF flow cytometry
- B:** HMs involving the peripheral blood are much more prevalent than those involving the central nervous system
- C:** Not only does peripheral blood generally have much higher cell counts, thus rendering cellularity criteria similar to those used for CSF meaningless, but also the many parameters associated with complete blood counts with differentials (CBC/diff) demonstrate substantial variance within a healthy population as compared to normal variation in CSF cell counts
- D:** None of the above

Question 4: Which of the following are **TRUE** regarding a negative PBFC result?

- A:** It was defined as one with no abnormal findings or with findings of limited clinical significance
- B:** It was defined as one with an abnormal blast or lymphoid population (B or T cell)
- C:** The findings for this result included low-count monoclonal B-cell lymphocytosis (absolute cell count $<500/\mu\text{L}$) and small circulating myeloid blast populations consistent with bone marrow recovery after treatment in patients with a history of HM
- D:** None of the above

Question 5: Which of the following are **CORRECT** regarding the results of the training cohort?

- A:** Within the cases classified as having a positive PBFC result, half had an abnormal lymphoid population and more than half had an abnormal blast population
- B:** Within the cases classified as having a negative PBFC result, the majority had no abnormal populations
- C:** Positive PBFC results were seen in 58% and 21% of cases with and without a history of HM, respectively
- D:** The parameters that showed the most significant difference between the positive and negative PBFC groups ten were % neutrophils, ALC, and % blasts/other cells

Question 6: Is it **TRUE** or **FALSE** that both models had a practical sensitivity of 100%?

- A:** TRUE
- B:** FALSE

Question 7: The decision tree proposed by *Healey et al.* evaluated several other serum laboratory values; the resulting decision tree showed that PBFC is mostly noninformative in?

- A:** Patients with an ALC less than 5.0×10^9 cells/L
- B:** Patients with an absolute monocyte count between 1 and 10%
- C:** Patients younger than 50 years
- D:** Patients 50 years or older with ferritin $450 \mu\text{g/L}$ or higher

Question 8: The models in this study improve upon previous methods by?

- A:** Including all PBFC cases sent for any indication
- B:** Including for the evaluation of acute leukemias and myeloid neoplasms
- C:** Operating on minimal clinical and CBC/diff data without the need for additional laboratory results
- D:** None of the above

Question 9: Which of the following are **TRUE** regarding the reflex protocol described by the authors?

- A:** It will be useful in instances when a PBFC order is placed in the absence of recent CBC/diff data
- B:** It will assure clinicians that further evaluation will be performed in cases that warrant it without the patient having to return for an additional blood draw
- C:** It will be useful in screening a general population
- D:** It could possibly save money

Question 10: Which of the following are **TRUE**?

- A:** A strength of this study is that the models are applicable in patients for whom only PBFC—but no CBC/diff—is ordered
- B:** A limitation of this study is that the cohort is from a single academic tertiary-care level institution with patient populations, clinician ordering practices, and specific flow cytometry analysis protocols that may differ from other institutions and facilities
- C:** A strength of this study is that an implemented improvement to the test ordering system is the creation of an order panel for PBFC that automatically includes a CBC/diff
- D:** A strength of this study is that this protocol is suitable for implementation at other institutions without prospective validation

END



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ANSWER SHEET

H2 (21)

Machine Learning Models Improve the Diagnostic Yield of Peripheral Blood Flow Cytometry

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