



Optimizing Markers for Chronic Lymphocytic Leukemia Diagnosis by Flow Cytometry: Results from the Nationwide Thai Lymphoma Study Group

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Introduction

- Accurate diagnosis of chronic lymphocytic leukemia (CLL) is crucial for effective management.
- Although recent advancements in flow cytometry have significantly improved diagnostic accuracy, distinguishing atypical cases of CLL remains challenging.
- This study aimed to identify specific markers to enhance CLL diagnosis and prognostic assessment.

Methods

- Blood or bone marrow from patients presenting with persistent lymphocytosis (>5 x10³/μL) was collected.
- Morphological, immunophenotypic, cytogenetic, and molecular analyses, along with clinical data, were collected to establish integrated diagnoses according to the WHO classification criteria. The Matutes score was calculated with a score of 3 or higher classified as CLL.
- Cases that initially met the diagnostic criteria but could not be definitively classified as CLL were categorized as non-CLL group.
- Flow cytometric analysis was performed using a customized CLL panel to evaluate the expression of CD markers for the diagnosis of CLL.

Results

- Recruitment was conducted across 12 centers under the Thai Lymphoma Study Group (TLSG) in Thailand between May 2018 and October 2023.
- A total of 228 patients were included in the study, including 206 patients (90.4%) in the CLL group and 22 patients (9.6%) in the non-CLL group.

Baseline Characteristics

- Baseline characteristics of patients in this study are summarized in **Table 1**.
- In the CLL group, the median age of participants was 65 years (interquartile range [IQR], 58-73 years), and 65.5% were male. The median white blood cell (WBC) count was 28.9 x10³/μL. RAI staging was 0 (25%), I (27.5%), II (22.1%), III (16.2%), and IV (9.3%).

Table 1 Baseline characteristics

	N=206
Age (years), median (IQR)	65.0 (58.0-73.0)
Sex	
- Female	71 (34.5%)
- Male	135 (65.5%)
Hb (g/dL), median (IQR)	12.2 (10.7-13.4)
WBC (x10 ³ /μL), median (IQR)	28.9 (17.1-53.4)
% Lymphocyte, median (IQR)	
Absolute lymphocyte count (x10 ³ /μL), median (IQR)	78.1 (68.2-86.0) 20.9 (11.8-44.1)
Platelet (x10 ³ /μL), median (IQR)	208.5 (155.0-269.0)
Uric acid (mg/dL), median (IQR)	6.0 (5.1-7.6)
LDH (IU/L), median (IQR)	237.0 (186.0-306.0)
B2-microglobulin (mg/L)	
- >3.5 mg/L	16 (42.1%)
- ≤ 3.5 mg/L	22 (57.9%)
RAI stage (0-IV)	
- 0	51 (25.0%)
- I	56 (27.5%)
- II	45 (22.1%)
- III	33 (16.2%)
- IV	19 (9.3%)
Relapse	54 (26.2%)
Death	27 (13.1%)

Diagnostic performance of biomarkers

- Flow cytometry results showed that CD5, CD19, and CD200 demonstrated sensitivities of 95.6%, 99.5%, and 88.6%, respectively.
- The positive predictive values (PPV) were 91.6%, 93.6%, and 97.8% for CD5, CD19, and CD200, respectively. Of note, both CD31 and CD11c, also showed high PPVs of 92.1%.

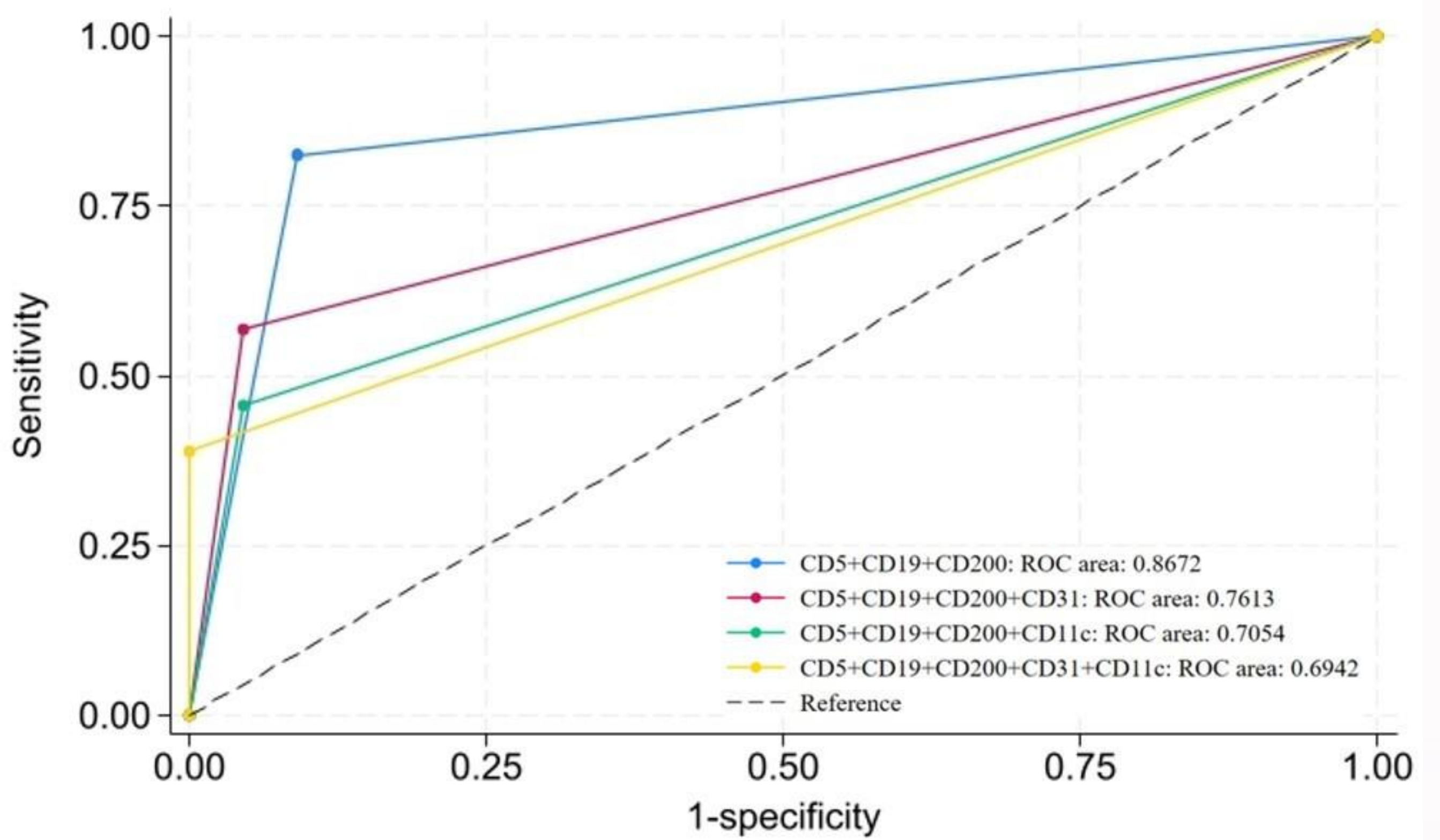
Model of diagnostic markers

- We combined markers with strong diagnostic performance, including CD5 and CD200 from the modified Matutes CLL scoring system, both of which showed high PPVs in our study, along with CD19, which had a PPV of 93.6%, to assess diagnostic accuracy for CLL in statistical models.
- Combining CD5, CD19, and CD200, the sensitivity and specificity for CLL diagnosis was 82.5% (95% CI, 76.6-87.4) and 90.9% (95% CI, 70.8-98.9), respectively. Given the high PPVs of CD31 and CD11c (92.1% each), these markers were incorporated into additional models.
- In the final predictive model, which incorporated five markers (CD5, CD19, CD200, CD31, and CD11c), both specificity PPV for CLL diagnosis were close to 100% (95% CI, 84.6-100 and 95.5-100, respectively).
- Detailed results of the predictive models, including AUC data, are shown in **Table 2** and **Figure 1**.

Table 2 Model of diagnostic markers

	Sensitivity	Specificity	PPV	NPV	Odds ratio
Model 1 CD5 + CD19 + CD200	82.5 (76.6-87.4)	90.9 (70.8-98.9)	98.8 (95.9-99.9)	35.7 (23.4-49.6)	47.22 (10.56-211)
Model 2 CD5 + CD19 + CD200 + CD31	56.8 (49.7-63.7)	95.5 (77.2-99.9)	99.2 (95.4-100)	19.1 (12.2-27.7)	27 (3.64-209)
Model 3 CD5 + CD19 + CD200 + CD11c	45.6 (38.7-52.7)	95.5 (77.2-99.9)	98.9 (94.3-100)	15.8 (10-23.1)	17.6 (2.33-133)
Model 4 CD5 + CD19 + CD200 + CD31 + CD11c	38.8 (32.1-45.9)	100 (84.6-100)	100 (95.5-100)	14.9 (9.6-21.6)	NA

Figure 1 Area under the curve of the predictive models



Parameters associated with relapse rate

- CD38 was identified as an independent predictor of increased relapse risk (aHR 2.73, 95% CI 1.40-5.32, p = 0.003), while leukocyte-associated immunoglobulin-like receptor (LAIR) expression was associated with a reduced relapse rate (aHR 0.34, 95% CI 0.17-0.67, p = 0.002).
- Regarding mortality rate, Hb < 10 g/dL and LDH ≥ 300 IU/L were significantly associated with increased mortality (aHR 6.02, 95% CI 1.42-25.53, p = 0.02 and aHR 3.0, 95% CI 1.29-6.96, p = 0.01, respectively).

Conclusion

- Expression of CD5, CD19, and CD200 by flow cytometry demonstrated a highly sensitive and accurate diagnostic utility for CLL. Adding both CD31 and CD11c to the predictive model further improved diagnostic specificity, reaching as high as 100%.
- The identification of relapse-associated markers provides valuable insights for personalized treatment strategies.