



CD200, CD79b, CD200:CD79b diagnostic value in CLL

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Abstract

Flow Cytometry is involved in the initial diagnostic work-up of CLL and other mature B-cell neoplasms (BCN), by immunophenotyping of the B-clonal population(s). Immunophenotyping of the clonal population is done by identification of CD markers and other extracellular and intracellular antigens. Chronic lymphocytic leukemia (CLL) is a BCN that relies heavily on flow cytometry for diagnosis, due to limited or no concrete cytogenetics or molecular abnormality unlike MCL whose translocation is well defined and documented. A retrospective study was conducted on patient samples collected at a metropolitan flow cytometry clinical department, to assess the sensitivity, specificity, and cutoff values of CD marker CD200, CD79b, and CD200:CD79b ratio for CLL. CLL has extensive research demonstrating high expression of CD200 and low expression of CD79b. 52 CLL cases were compared to 54 non-CLL cases to determine diagnostic cut-off values. CLL was also compared to a mantle cell lymphoma stand-alone category, and same CD marker characteristics were defined as mentioned above. A scoring system was developed based on the cut-off values of the three variables and was tested against 50 blind samples, clinical sensitivity and specificity were then calculated. CD200, CD79, and CD200:CD79b are markers that are effective and efficient for categorizing B-cell clonal populations, as they demonstrate statistically good sensitivity and specificity for CLL in distinguishing from other MBN. The CD200:CD79b ratio application has good predictivity value for comparison between CLL vs non-CLL and for CLL vs MCL.

Background

B-cell lymphoproliferative disorders (BCLD) can be characterized using flow cytometry by generating an immunophenotype profile of the clonal population. Immunophenotyping can aid in the diagnostic evaluation of malignancy as it provides insight to the aberrant expression, the over-expression or under-expression of other characteristic B-cell CD markers. Chronic lymphocytic leukemia (CLL) is well characterized BCLD, typically CD5+ and CD10- and is positive for known B-cell markers including CD19, CD20, and CD22.

CD79b is a subunit of CD79 and is expressed on immature B-cells before expression of CD20 and heavy chain rearrangement, expression ceases during plasma cell differentiation. CD79b expression is decreased in CLL, and it is estimated that about 85% of CLL/small lymphocytic leukemia (SLL) have genetic modifications of the CD79b gene in the transmembrane and cytoplasmic domain. CD200 is a transmembrane type-1 glycoprotein. Glycosylation differs in the different cell types expressing CD200 which include dendritic cells, epithelial cells, activated B & T-cells, and endothelial cells, no functional differences have been attributed to these modifications.

CD markers have been used for the development of scoring systems that aim to categorize BCLD's, even in cases of atypical phenotypes. Matute's 1975 scoring system is often cited and modifications to the original scoring system, another scoring system is the CLLflow score. Research on two marker combinations is limited, but research has been done on CD160 and CD200 and its application in differentiating between CLL and other mature B-cell neoplasms (MBN). This study will focus on CD200, CD79b, and the ratio of CD200:CD79b in distinguishing between CLL and non-CLL cases, as well as how these variables perform in distinguishing CLL from MCL.

Methods

Samples were processed in a large metropolitan hospital flow cytometry department from March 2021 to September 2024. Table 1 lists the diagnosis of the 107 cases included. All samples underwent a B1 screening panel to identify the presence or absence of a B-cell clonal population. The sample reflexed to a B2 panel consisting of markers used to assist in the categorization of any B-cell neoplasm. MCL and CLL cases were included if cytogenetics or molecular testing confirmed the diagnosis. The finding of the t(11;14) in peripheral blood and in another site was the confirmatory criteria for MCL. CLL confirmatory criteria consisted of the detection of common mutations associated with this disease, which includes deletion of 11q22.3, deletion of 13q14.3 and trisomy 12.

CLL peripheral blood and bone marrow specimens were analyzed using BD FACSLyricTM Cell Analyzer (Becton, Dickinson, Milpitas, California) for immunophenotyping. Specimens underwent red cell lysis with ammonium chloride and incubation with B1 or B2 antibody cocktail (Table 2) Original-screening panel results for the B2 tube were re-gated using WinList (Verity Software House, Lexington, KY). A protocol bundle was then created for analysis using 2-parameter histograms and the mean fluorescence intensity (MFI) of CD79b and CD200 (Figure 1) which was used to calculate the CD200:CD79b ratio. A receiving operating curve (ROC) analysis (Table 3) was performed using EP evaluator (Data Innovations, Colchester, VT) to determine sensitivity, specificity, AUC (area under the curve) and diagnostic cut off values for CD200, CD79b, and ratio CD200:CD79b to aid in the differentiation of CLL from non-CLL cases and CLL from MCL. Cut-off values generated by the ROC analysis to test efficiency, utilizing a 95% CI, CD200, CD79b and CD200:CD79b MFIs were used to create a scoring system to test and diagnose 50 blind sample cases as either non-CLL or CLL. One point was given if the MFI meet the criteria for each of the respective CD marker: CD76b was < 227.16, CD200 was ≥ 1135.83, CD200:CD79b was ≥ 3.9431. If the case scored 0/3 or 1/3 the case was designated as non-CLL, if the case scored 2/3 or 3/3 the case was designated as CLL. The scored diagnosis was then compared to the actual diagnosis of the blind case, as a final step the clinical specificity and sensitivity was calculated for the scoring system.

Results

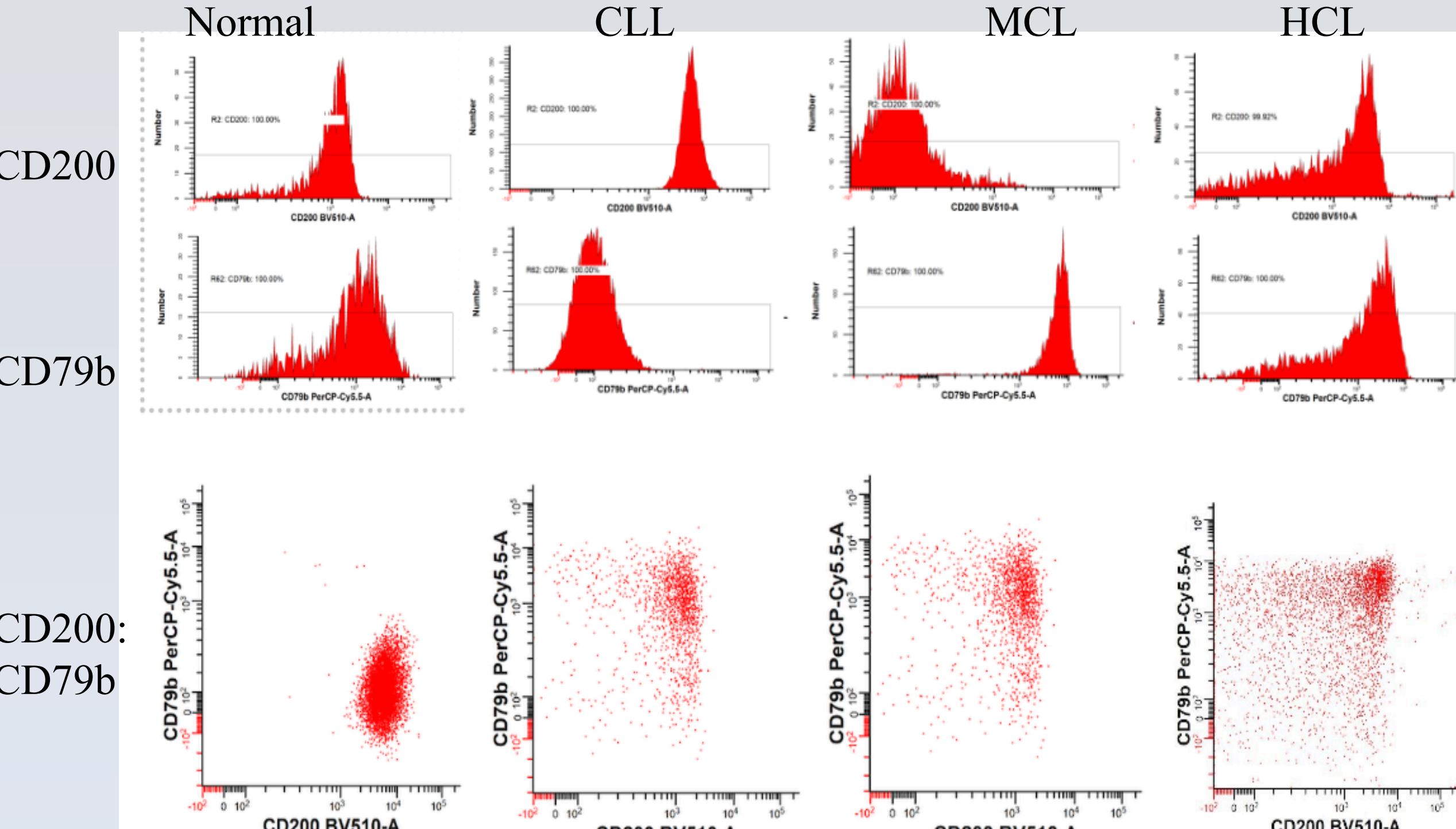


Figure 1: Flow cytometry two-parameter scatter plots were used to generate the MFI of CD79b, CD200, and CD200:CD79b ratio. A Mann-Whitney test for CD200 MFI had a U=178, Z= -7.7449, P <0.0001, CD79b had a U=2688, Z=8.1114, p <0.0001, MFI of CD200:CD79b had a U=64, Z=-8.4653, p <0.0001.

Table 1: Diagnosis of cases used in this study

| Diagnosis | Number of Cases |
|---|-----------------|
| Chronic Lymphocytic Leukemia (CLL) | 52 |
| Non-CLL Cases | 54 |
| Mantle Cell Lymphoma (MCL) | 24 |
| B-cell Acute Lymphocytic leukemia (B-ALL) | 1 |
| Marginal Cell Lymphoma (MZL) | 7 |
| Hairy Cell Leukemia (HCL) | 2 |
| Lymphoplasmacytic lymphoma | 2 |
| Diffuse Large B-cell lymphoma | 2 |
| Non-MBN | 10 |
| Total: | 107 |

Table 3: ROC Analysis Data

| | Specificity | Sensitivity | AUC | Cut-off Value |
|------------------------|-------------|-------------|--------|---------------|
| CLL vs. non-CLL | | | | |
| CD200 | 79.6% | 94.2% | 0.9336 | ≥ 1135.83 |
| CD79b | 90.4% | 90.7% | 0.9772 | < 227.16 |
| CD200:CD79b | 92.6% | 98.1% | 0.9772 | ≥ 3.9431 |
| CLL vs. MCL | | | | |
| CD200 | 100% | 100% | 1 | ≥ 687.16 |
| CD79b | 87.0% | 89.1% | 0.9699 | < 439.99 |
| CD200:CD79b | 100% | 98.1% | 0.933 | ≥ 3.9431. |

The MFI for CD200, CD79b and CD200:CD79b were recorded for each clinical case. Figure 1 provides a comparison of CD79b, CD200, and CD200:CD79 MFI across the different categories, after re-gating of the original B2 panel for better isolation of the B-cell clonal population. The distribution of the three variables demonstrated a skewed distribution. A Mann-Whitney test was then performed. Non-CLL and CLL populations were found to be statistically different in relation to the MFI of CD200, CD79b, and CD200:CD79b. The Dixon-outlier test was performed on MCL case H23-007500 for the CD200:CD79b ratio and was found to be >1/3 of the range, identifying this case as an outlier

Discussion

The focus of this study was the performance of the three CD markers in distinguishing between CLL and non-CLL, in which MCL was compared in a stand-alone category as well as a part of the non-CLL group. A limitation of this study was a small sample size partly due to the nature of flow cytometry and lab volumes in just one clinical lab location. In the context of CLL vs non-CLL, the CD200:CD79b ratio had a higher sensitivity and specificity than just the stand-alone CD markers. In the context of CLL vs MCL, CD200 scored 100% for both measures. CD200:CD79b in the context of CLL vs MCL, was still as specific and with higher sensitivity than the non-CLL category. The clinical specificity and sensitivity were both 100%. The scoring system is useful in calculating a combination of phenotypic changes in CD200, CD79, and CD200:CD79b for CLL. Clinical specificity and sensitivity are important measures, due to the following ancillary testing and follow-up that can occur to the patient given a false negative or false positive result.

A scoring system based on CD200, CD79b, CD200:CD79b suggests good clinical sensitivity and specificity, demonstrating the ability to detect a clinical condition (CLL) and score low or negative when the clinical condition is absent, respectively. A combination or ratio of CD200:CD79b demonstrated comparable or better performance than the stand-alone markers and had a cut-off value that was stable across the two different conditions tested, illustrating a stable or reliable marker. Research in CD marker ratio is extremely limited but provides a potential avenue for research as is the case with CD200:CD79b and its ability to differentiate between CLL and non-CLL cases within this framework. Addition of this ratio if reflex or B-cell disorder panel could aid in the categorization of the B-cell clonal population in conjunction with other flow cytometry results, while awaiting additional or

Table 2: Antibodies included in each panel.

| B1 | B2 |
|--------|--------|
| Kappa | CD19 |
| Lambda | CD20 |
| CD3 | CD22&2 |
| CD5 | CD23 |
| CD19 | CD43 |
| CD20 | CD45 |
| CD45 | CD79b |
| CD45 | CD103 |
| | CD200 |

