The contribution of CD200 to the diagnostic accuracy of Matutes score in the diagnosis of chronic lymphocytic leukemia in limited resources laboratories

Sana Dlawar Jalal*
Department of Pathology, College of Medicine, University of Sulaimani, Sulaimani, Iraq
* dr.sanajalal612@gmail.com

Abstract
Flow cytometry immunophenotyping has an essential role in distinguishing chronic lymphocytic leukemia from other B-chronic lymphoproliferative disorders. Recently, CD200 is considered as a relatively consistent marker in chronic lymphocytic leukemia. We retrospectively assessed CD200 expression in 252 patients with B chronic lymphoproliferative disorders with four-color flow cytometry. CD200 expression estimation included the proportion of positive cells (≥30%) and the mean fluorescence intensity ratio. Additionally, we have incorporated CD200 into Matutes score, also replaced FMC7 and CD79b in an attempt to improve the score discriminative power. Of 252 patients enrolled, 199(79%) patients were classified as chronic lymphocytic leukemia and 53 (21%) as other B-chronic lymphoproliferative disorders. All chronic lymphocytic leukemia cases and 20 of 53 (37.7%) of other B-chronic lymphoproliferative disorders demonstrated high CD200 expression (≥30%). Further, CD200 (≥30%) revealed a higher accuracy in comparison to other markers in Matutes score (range: 51%–92.5%). Also, CD200 addition to the Matutes score has correctly recognized all 199 chronic lymphocytic leukemia cases including 10 atypical chronic lymphocytic leukemia cases. As for non-CLL cases, 20 of 53 attained a higher score, yet keeping the original diagnosis. Moreover, CD200 enhanced the diagnostic accuracy of Matutes score to 100%, and when included in a simplified 4-markers score, showed an accuracy of 99.8% compared to 99.4% of Matutes score. In conclusion, CD200 is an accurate diagnostic marker for chronic lymphocytic leukemia, and can refine the modified Matutes score accuracy when added with other markers.

Introduction
Chronic lymphocytic leukemia (CLL) is a clonal expansion of monomorphic, mature, immunologically incompetent CD5+ B-cells in peripheral blood, bone marrow, and secondary lymphoid organs. It’s considered the most common leukemia in adults with a male predominance and an average age at presentation of 72 years. The incidence is 10 to 20 times less in Asia than in Western countries suggesting the influence of some genetic and environmental factors on disease susceptibility [1,2]. Diagnostic accuracy is of utter importance as the therapeutic
options differ significantly for various B-chronic lymphoproliferative disorders (B-CLPD). Chronic lymphocytic leukemia diagnosis requires the presence of \( \geq 5 \times 10^9/l \) monoclonal B-cells exhibiting characteristic immunophenotype initially described by Estella Matutes’ Matutes scores MS” [3] [surface membrane immunoglobulin (SmIg) weak, CD5+, CD19+, CD23+, CD22 weak/–, and FMC7 –], that was modified later by replacing CD22 by CD79b [4]. In Matutes scoring system, a value of 0 or 1 is assigned according to the expression of the abovementioned five markers. The majority of CLL cases have a score of 4 or 5 whereas non-CLL cases score below 4. Some atypical CLL cases may score 3 [5,6] where the addition of more markers such as ROR1 [7], CD81 [8], CD43 [9], and most importantly CD200 would be very helpful [10]. CD200, a glycoprotein on the surface membrane of normal B-cells, B-cell precursors, some T-cells, dendritic cells, and neurons [11], was first described in 2009 for being uniformly expressed in CLL, but absent in mantle cell lymphoma (MCL) [12]. Since then, it’s potential role had expanded and considered as valuable as CD23 in the diagnosis of B-CLPD [13]. This study, therefore, has investigated the diagnostic benefit of adding CD200 to MS in a large Iraqi single-center cohort of 252 patients with chronic B-cell lymphoproliferative disorders. Further, we assessed the performance of the 4-marker score; CD5/CD23/CD200/sIgM, in optimizing the accuracy of CLL diagnosis in resource constrained laboratories.

**Materials and methods**

**Patients**

The current retrospective analysis started on 12/1/2020 including a total of 195 peripheral blood and 57 bone marrow aspirates and/or bone marrow biopsies of patients with suspected B-CLPD processed at Sulaimani Public Health Laboratory, Sulaimani-Kurdistan/Iraq over the period from January 2012 to December 2019. The patients aged between 35-87 years old with a median of 63 years, 184 (73%) were males and 68 (27%) were females, with a male-to-female ratio of 2.7:1. All enrolled cases were referred from hemato-oncology hospitals from different provinces in Iraq and the study sample can be considered representative of a larger population. The diagnosis was determined by results from diagnostic procedures including; cytomorphology, flow cytometry (FC), and immunohistochemistry (IHC) according to the World Health Organization (WHO) guidelines and International Working Group on CLL (IWCLL) [14,15]. The inclusion criterion was as follows; Patients with full database; demographic and laboratory data including a set of flow cytometry markers necessary for B-CLPD diagnosis.

**Immunophenotypic analysis**

All specimens, freshly collected in K3 EDTA tubes, were stored at 4°C, stained and analyzed within 24–36 hours from collection using a direct immunofluorescence method, as detailed below: A total of \( 1 \times 10^6 \) cells from whole blood samples were incubated for 15 minutes in the dark at 37°C with monoclonal antibodies (MAbs) targeting the antigens: CD5, CD23, CD19, CD20, CD10, FMC7, CD79b, CD200, sIgM, Kappa, Lambda, CD38, CD103, CD11c, CD25, and CD123. Isotype antibodies were used as a negative control in separate tubes. All MAbs were purchased from BD Biosciences (Fluorochromes and clones are outlined in S1 Table). Cells were lysed within 5 minutes by (Becton Dickinson (BD) FACS Lysing solution and washed in Phosphate Buffer Solution (PBS). Thereafter, cells were re-suspended in 500 µL of PBS and immunophenotyping analysis was performed using two lasers, four-color, six-parameter BD FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA). Data acquisition and analyses were performed using CellQuest Pro software (BD Biosciences). Calibration was performed using BD CaliBRITE beads with daily quality control to preserve the reproducibility of the fluorescence intensities. At least 10,000 gated events were acquired and the lymphocytes
population was selected by gating on CD45\textsuperscript{high}/sideways scatter (SSC)\textsuperscript{low}, and subsequent analysis was carried out on CD19+ cells. The modified Matutes score was calculated as described earlier [4], and positivity was established as $\geq$ 30% positive cells population [6,16–18]. CD5 and CD23 were counted score 1 when the positive cells population was $\geq$ 30%, while FMC7 and CD79b were considered score 1 when the positive cells population was <30%. Additionally, slgM was considered score 1 when the expression was weak. Typical CLL cases were defined by a score $\geq$ 4 and atypical cases were identified by a score < 4. Likewise, CD200 was designated score 1 when the positive cells population was $\geq$ 30%. CD200 expression intensity was estimated as mean fluorescence intensity ratio (MFIR) (MFI sample/MFI isotype) with a positivity threshold set up at 18 according to a prior study [6] (S1 Fig). Furthermore, the expression pattern (weak, moderate, or high) was evaluated according to the log scale of the fluorescence axis on the B-cell population. Accordingly, weak expression if the positive peak lied within the first logarithmic percentile, moderate in the second percentile, and strong when it was above the second percentile [19].

**Ethical consideration**

The study was approved by the ethical committee at Sulaimani College of Medicine, University of Sulaimani, Iraq. All methods were performed in accordance with the Helsinki Declaration and verbal informed consents were obtained from all enrollees and documented within laboratory database.

**Statistical analysis**

SPSS version 25.0 (Armonk, NY: IBM Corp, USA) was used to carry out the analysis. For evaluating the performance of diagnostic tests and the accuracy of a statistical model, the sensitivity and specificity were measured using the ROC test, and optimal cut-values have been chosen as to achieve a maximum sensitivity and specificity. The sensitivity and specificity of CD200, as a percentage of positive cells, were calculated at an optimum cut value of 88%, while the cut value for CD200 MFIR was 36.8%. A score of $\geq$4 was selected to evaluate CLL diagnosis in the classical and currently modified Matutes Scores, and MS was considered as a comparator with the modified MS. McNemar’s test was used to estimate statistically significant differences between MS systems. Additionally, continuous variables were compared using Mann–Whitney’s U test. $P$ values of $0 < 0.05$ were considered statistically significant.

**Results**

The immunophenotypic classification of 252 patients with a provisional diagnosis of B-CLPD is as follows: 199 cases classified as CLL (79%), and 53 cases were non-CLL (21%) [20 mantle cell lymphoma (MCL), 17 marginal zone lymphoma (MZL),8 hairy cell leukemia (HCL),3 follicular lymphomas (FL)and 5 other B-CLPD [2 lymphoplasmacytic lymphomas (LPL),1 Waldenström macroglobulinemia (WM), and 2 prolymphocytic leukemia (PLL)], (Table 1).

| Patients (n = 252) | No. | %   |
|-------------------|-----|-----|
| CLL               | 199 | 79  |
| Mantle cell lymphoma (MCL) | 20 | 7.9 |
| Marginal zone lymphoma (MZL) | 17 | 6.7 |
| Hairy cell leukemia (HCL) | 8 | 3.2 |
| Follicular lymphoma (FL) | 3 | 1.2 |
| Other B-CLPD      | 5  | 2   |

Table 1. Diagnosis of 252 cases with suspected B-chronic lymphoproliferative disorders.

https://doi.org/10.1371/journal.pone.0247491.t001
Of the total CLL cases, 189 / 199 CLL cases had the typical CLL phenotype (MS ≥4), while 10 cases scored 3 and diagnosed as atypical CLL (with negative cyclin D1 by IHC on LN biopsy or BM biopsy). Details of atypical CLL cases are provided in (S2 Table). All the B-CLPD cases with MS between 0–3 required correlation with clinical data and another pathology testing including, histological and where available IHC. Cases with typical morphology and immunophenotypic markers expression (e.g. CD103, CD25, CD11c, and CD123) were regarded as convenient for HCL diagnosis without further histopathological confirmation. Regarding non-CLL B-CLPD, 46/53(86.8%) had MS of 0–2 (Table 2).

In CLL cases, CD200 positive B- cells (> 30%) were detected in all cases [median 97.9% (50.6–100)] as opposed to a highly heterogeneous CD200 expression pattern in the non-CLL group, reported in 37.7% of cases, [median 9.8 (0.10–91.8%)], p value<0.0001 (Table 2 and Fig 1). Among non-CLL cases, the highest CD200 values were seen in MZL (median 55.9%), followed by HCL (median 46.4%), while all MCL cases were negative for CD200. Similarly, all

Table 2. CD200 expression in B-cell chronic lymphoproliferative disorders.

|                | Median percentage (range) | n, CD200 ≥ 30% | Median MFIR (range) | n, CD200 MFIR ≥ 18 | n, CD200 MFIR < 18 | Matutes score (n patients) |
|----------------|---------------------------|-----------------|---------------------|-------------------|-------------------|---------------------------|
| CLL (n = 199)  | 97.9(50.6–100.0)          | 199(100%)       | 0                   | 199 (100%)        | 0                 | 3(10), 4(95), 5(94)       |
| HCL (n = 8)    | 46.4 (0.9–91.8)           | 5 (62.5%)       | 3 (37.5%)           | 5 (62.5%)         | 3 (37.5%)         | 0(1), 1(3), 2(3)          |
| MZL (n = 17)   | 55.9 (0.4–88.0)           | 12 (70.6%)      | 5 (29.4%)           | 12 (70.6%)        | 5 (29.4%)         | 0(7), 1(4), 2(4), 3(2)    |
| MCL (n = 20)   | 2.3 (0.1–41.0)            | 0               | 20 (100%)           | 0                 | 20 (100.0%)       | 1(10), 2(7), 3(3)         |
| FL (n = 3)     | 1.7 (0.9–70.6)            | 1 (33.3%)       | 2 (66.7%)           | 1 (33.3)          | 2 (66.7%)         | 2(2), 3(1)                |
| Others (n = 5) | 5.9 (1.9–57.6)            | 2 (40.0%)       | 3 (60.0%)           | 2 (40.0%)         | 3 (60.0%)         | 0(1), 1(1), 2(3)          |

https://doi.org/10.1371/journal.pone.0247491.t002

Fig 1. CD200 expression levels (percent of positive cells and MFIR) on 252 B-cell chronic lymphoproliferative disorders enrolled in this study.

https://doi.org/10.1371/journal.pone.0247491.g001
199 CLL cases displayed a potentially higher MFIR of CD200 expression [median MFIR of 59 (39.3–69.9) versus 8.7 (0.7–77) in non-CLL group (P < .0001)] (Table 2 and Fig 1). Further, CD200 expression (MFIR ≥ 18.0); was seen in all cases of CLL and in 20/53 (37.7%) cases from non-CLL group; including 5 of 8 HCL cases and 12 of 17 MZL but non from MCL group. Additionally, atypical CLL group displayed less intense CD200 expression opposed to classical CLL; as proportion of positive cells ≥ 30% [median 62.6(50.6–84.7%)] vs. [median 98 (88.9–100)] and MFIR [median 50.3 (40–57)] vs median 59.8(39.3–69.9)], respectively, p value <0.001. Of note, a higher CD200 expression (CD200 ≥30% and MFIR) correlated significantly to higher MS scores p <0.0001 (Fig 2A and 2B).

**Diagnostic utility of CD200 in CLL diagnosis**

As a diagnostic marker, CD200 (≥30%) revealed a sensitivity of 94.97%, a specificity of 98.11%, with considerably higher accuracy than other MS markers (CD5, CD23, FMC7, CD79b and sIgM) (99.1%) in CLL diagnosis, p value <0.001, (Table 3). This is at variance to CD200 expression evaluated as MFIR (≥18.0), where a lower accuracy (93.6% vs. 99.11%, respectively), a higher sensitivity (100% vs. 94.97%, respectively), and lower specificity (83.2% vs. 98.11%, respectively).

| Marker | Matutes Score | Sensitivity % (95% CI) | Specificity % (95% CI) | CLL vs. non-CLL % (95% CI) | P value |
|--------|---------------|-------------------------|------------------------|-----------------------------|---------|
| CD200a | Negative 0    | positive 1              | 94.97 (91.0 - 97.6)    | 98.11 (89.9 - 100.0)        | 99.1 (96.9–99.9) | <.001 |
| CD200b | Negative 0    | positive 1              | 100.0 (98.2 – 100.0)   | 83.02 (70.2 - 91.9)         | 93.6 (89.8–96.3) | <.001 |
| CD5a   | Negative 0    | positive 1              | 100.0 (98.2–100.0)     | 62.26 (47.9–75.2)           | 81.1 (75.7–85.8) | <.001 |
| CD23a  | Negative 0    | positive 1              | 98.49 (95.7–99.7)      | 79.25 (65.9–89.2)           | 88.9 (84.3–92.5) | <.001 |
| FMC7c  | Negative 1    | positive 0              | 93.97 (89.7–96.8)      | 20.75 (10.8–34.1)           | 57.4 (51.0–63.5) | <.05  |
| CD79bc | Negative 1    | positive 0              | 52.26 (45.1–59.4)      | 58.49 (44.1–71.9)           | 55.4 (49.0–61.6) | NS    |
| sIgMd  | Weak 1        | Moderate/Strong 0       | 87.44 (82.0–91.7)      | 52.83 (38.6–66.7)           | 70.1 (64.1–75.7) | <.001 |

*aCD200 was regarded score 1 when the positive B -cell population was ≥30%.

bCD200 was regarded score 1 when the positive B-cell population was MFIR ≥18.

cFMC7 and CD79b were regarded score 1 when the positive B-cell population was <30%.

d sIgM was regarded score 1 when the expression was weak.

NS = Non Significant P > 0.05.
vs. 98.1%, respectively) were detected with a significant difference in CLL discriminative accuracy, \( p < 0.04 \). Expectedly, CD5, and CD23 were informative in this analysis, in contrast to FMC7 and CD79b markers that didn’t yield a high diagnostic value. Likewise, sIgM showed a low specificity in CLL diagnosis (Table 3).

**Modification of Matutes score**

The addition of CD200 (\( \geq 30\% \)) to MS has modified the score in 30 of 252 cases included in the study. All 10 cases of atypical CLL (MS 3) were re-categorized into MS 4 (i.e., classical CLL). Likewise, 20 cases in the non-CLL group had a modified higher score; including 1 HCL case (from MS 3 to MS 4) and 9 from non-CLL cases into MS 3 (4 from MZL, 2 HCL, 2 PLL and 1 from FL), (Table 4). Also, the simple four-marker MS including CD200 together with CD5, CD23 and sIgM, has categorized 94% of CLL cases as classical CLL (\( \geq 4 \)), whereas 12 cases have a MS of 3. Additionally, 4 cases from other B-CLPD (2 MZL, and 2 PLL cases) scored 3 in the 4-marker scoring system.

Interestingly, the addition of CD200 to CD5, CD23, CD79b, FMC7, and sIgM has improved the accuracy and sensitivity of the MS from 99.4% to 100% and 94.97% to 100%, respectively, while keeping a high specificity (Table 5). Furthermore, a high diagnostic accuracy was revealed when sIgM was replaced by CD200. Similarly, the substitution of FMC7 and CD79b by CD200 in a simplified 4-marker MS has slightly refined the accuracy of MS to 99.8% while keeping high sensitivity and specificity (Table 5).

**Discussion**

Flow cytometry immunophenotyping is crucial for the diagnostic workup of B-CLPD due to its cost-effectiveness and capability of characterizing multiple parameters simultaneously that may enhance the accuracy of flow cytometry-based diagnosis [20]. In the current cohort, we have assessed the value of CD200 in the differential diagnosis of different B-chronic lymphoproliferative disorders and whether it adds a discriminative potential to MS. Although the

| Table 4. Patients classification according to Matutes score and the alternative proposed score. |
|-----------------------------------------------|
| **Classical MS** | **MS with CD200 added** | **MS with CD200 replaced FMC7 & CD79b** |
| **MS** | **CLL** | **Non-CLL** | **CLL** | **Non-CLL** | **CLL** | **Non-CLL** |
| \( \geq 5 \) | 94 | 0 | 189 | 0 | 0 | 0 |
| 4 | 95 | 0 | 10 | 1 | 187 | 0 |
| 3 | 10 | 7 | 0 | 16 | 12 | 4 |
| 2 | 0 | 19 | 0 | 19 | 0 | 16 |
| 1 | 0 | 18 | 0 | 14 | 0 | 30 |
| 0 | 0 | 9 | 0 | 3 | 0 | 3 |

https://doi.org/10.1371/journal.pone.0247491.t004

| Table 5. Sensitivity, specificity, and accuracy of Matutes scoring systems CLL versus Non-CLL differential diagnosis. |
|-----------------------------------------------|
| **Scoring system** | **Sensitivity % (95% CI)** | **Specificity % (95% CI)** | **CLL vs. Non-CLL % (95% CI)** |
| CD5, CD23, FMC7, sIgM, CD79b | 94.97 (91.0 - 97.6) | 100.0 (92.3 - 100.0) | 99.4 (97.4 – 100.0) |
| CD5, CD23, FMC7, sIgM, CD79b, CD200 | 100.0 (98.2 – 100.0) | 98.04 (89.6 - 100.0) | 100.0 (98.4–100.0) |
| CD5, CD23, sIgM, CD200 | 93.97 (89.7 - 96.8) | 100.0 (93.0 - 100.0) | 99.8 (98.1–100.0) |

https://doi.org/10.1371/journal.pone.0247491.t005
latter score has been essential for the diagnosis of CLL, in some cases, the diagnosis might be challenging based on the five markers included, which is particularly featured by “atypical CLL” with MS of 3 \[\text{[21,22]}\]. This would justify the addition of further markers such as CD200, or relying on further evaluation (i.e., FISH) and clinical decision in atypical cases \[\text{[22–24]}\]. While CD200 evaluation is not required in the CLL diagnostic criteria by the World Health Organization \[\text{[14]}\], it was implemented in the EuroFlow guidelines and earlier research has declared it’s a high diagnostic value \[\text{[25]}\].

In concordance with previous results, CLL cases in this cohort showed a consistent manner of CD200 positivity together with the 10 cases of the atypical phenotype (MS < 4), though with lower intensity levels \[\text{[5,23,26]}\]. Additionally, CD200 expression was significantly higher in CLL than in other B-CLPD in agreement with others \[\text{[10,12,16,17,21]}\]. We have demonstrated that all MCL cases enrolled were negative for CD200 in agreement with most previous studies \[\text{[13,16,27–29]}\], while others reported a dim expression of CD200 in some MCL \[\text{[5,6]}\]. Also, we have illustrated that CD200 is expressed in other non-MCL B-CLPD, yet in a heterogeneous pattern, ranging from negative to moderate as featured by others \[\text{[6,10,29–31]}\].

The contribution of CD200 to modify the accuracy of MS has not been addressed in Iraq with few earlier international studies. A German group (Köhnke et al., in 2017) had incorporated the MFIR of CD200 positive cells into MS and proposed a new score called “CLLflow Score,” with a score above zero correlates with CLL diagnosis, whereas a score of zero or below is unlikely for classical CLL cases \[\text{[21]}\]. Likewise, two more recent studies (D’Arena et al., 2018) \[\text{[17]}\], and (Mora et al., 2019) \[\text{[6]}\] have revealed that CD200 ≥ 30% has a high sensitivity, specificity and high diagnostic accuracy. Moreover, when CD200 assessed with other MS markers or replaced one or more markers, an increased accuracy of the score was manifested. In agreement with previous studies, this study has displayed that CD200 (≥30%) incorporation into MS has refined its accuracy to (100%), and therefore, raised its robustness in CLL diagnosis \[\text{[6,17,21]}\]. It’s worthy to mention that although MFIR is a reliable marker of antigen expression level, and has shown a high diagnostic accuracy (though lower than CD200 ≥30% in the current study), is not easy to calculate in daily routine cases and not commonly documented in flow cytometry reports, hence not accessible for clinicians \[\text{[21]}\].

On the other hand, both CD79b and FMC7 have demonstrated a low performance in contrast to what has been described in earlier studies \[\text{[6,21]}\]. Such variations might be attributed to the study size, and whether a positive marker expression was considered as percentage of positive B-cell population (whether the cutoff point was 20% or 30%) or MFIR. Also if internal control or isotype control was regarded as the negative population in flow cytometry analysis. Finally, factors related to the antibodies comprising the B-CLPD panel, like antibodies conjugated fluorochromes, and their clones might possibly play a role too.

sIgM shown to be a sensitive yet not specific marker in CLL differentiation from other B-CLPD. This might be explained by an aberrantly low expression of sIgM in some B-CLPD with brighter sIgM expression in some CLL cases \[\text{[21]}\].

The classical MS in the current study has shown to be effective in separating CLL from other B-CLPD once the MS is high (≥4) or low (MS 0–1), while when MS value was between 2 and 3, atypical CLL (MS3,n = 10) overlapped with other B-CLPD scoring 3 (n = 7) (Table 4), \[\text{[32]}\]. The addition of CD200 to MS has reclassified the atypical CLL into classical CLL and hence allowed clear discrimination from other B-CLPD. Indeed, the MS modifications in this study have derived a high accuracy in CLL diagnosis. Additionally, the simplified four-marker MS has correctly identified 187/199CLL (94%) cases as classical scoring 4/4 with a high specificity and diagnostic accuracy \[\text{[17]}\], while 12 CLL cases scored 3/4 and hence diagnosed as atypical CLL on the basis of classical MS cutoff, the most reliable scoring system yet \[\text{[5]}\]. Likewise, 4 cases from other B-CLPD (2MZL, and 2PLL cases) have scored 3/4, though these cases
were morphologically distinct from CLL. Besides, all MCL cases scored 1-2/4 in the simplified score with no overlap with atypical CLL cases. Despite the fact that CD200 incorporation to classical MS in the current study has demonstrated 100% accuracy, the 4-marker MS would be much preferred in resource limited settings in an attempt to minimize the markers demanded for classical MS while keeping a high diagnostic performance.

In conclusion, CD200 is an accurate diagnostic marker that is consistently expressed in CLL cases and has potentially distinguished atypical CLL cases from MCL in this study. Furthermore, CD200 assessment within an MS seems to be suitable and convenient for laboratories when the antibodies supply is restricted, and cytogenetic tests are not readily available for challenging cases. Moreover, a cost-effective four -marker panel can be designed with improved accuracy compared to the classical MS. Finally, large prospective studies are prudent to analyze and validate diagnostic accuracy of 4-marker MS with an emphasis on the cutoff point that unequivocally distinguish CLL form other B-CLPD.

Supporting information

S1 Fig. Receiver operating characteristics for CD200 positivity as MFIR.
(TIF)

S1 Table. Supporting information regarding CD markers used at our study.
(DOCX)

S2 Table. Features of 10 atypical CLL cases.
(DOCX)

Acknowledgments

The deepest gratitude and appreciation for the patients who were enrolled in this study. We are also grateful to staff of flow cytometry department at Sulaimani Public Health Laboratory.

Author Contributions

Conceptualization: Sana Dlawar Jalal.

Data curation: Sana Dlawar Jalal.

Formal analysis: Sana Dlawar Jalal.

Investigation: Sana Dlawar Jalal.

Methodology: Sana Dlawar Jalal.

Resources: Sana Dlawar Jalal.

Software: Sana Dlawar Jalal.

Validation: Sana Dlawar Jalal.

Visualization: Sana Dlawar Jalal.

Writing – original draft: Sana Dlawar Jalal.

Writing – review & editing: Sana Dlawar Jalal.
References

1. Autore F, Strati P, Laurenti L, Ferraioli A. Morphological, immunophenotypic, and genetic features of chronic lymphocytic leukemia with trisomy 12: a comprehensive review. Haematologica. 2018; 103 (6):931–8. https://doi.org/10.3324/haematol.2017.186684 PMID: 29748447

2. Burger JA. Treatment of Chronic Lymphocytic Leukemia. N Engl J Med. 2020; 385):460–73.

3. Matutes E, Owusu-Ankomah K, Morilla R, Garcia Marco J, Houllihan A, Que TH, et al. The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. Leukemia. 1994; 8(10):1640–5. PMID: 7523797

4. Moreau EJ, Matutes E, A’Hern RP, Morilla AM, Morilla RM, Owusu-Ankomah KA, et al. Improvement of the chronic lymphocytic leukemia scoring system with the monoclonal antibody SN8 (CD79b). Am J Clin Pathol. 1997; 108(4):378–82. https://doi.org/10.1093/ajcp/108.4.378 PMID: 9322589

5. Ting YS, Smith S, Brown DA, Dodds AJ, Fay KC, Ma DDF, et al. CD200 is a useful diagnostic marker for identifying atypical chronic lymphocytic leukemia by flow cytometry. Int J Lab Hematol. 2018; 40(5):533–9. https://doi.org/10.1111/iijh.12857 PMID: 29806244

6. Mora A, Bosch R, Cuellar C, Vicente EP, Blanco L, Martino R, et al. CD200 is a useful marker in the diagnosis of chronic lymphocytic leukemia. Cytometry B Clin Cytom. 2019; 96(2):143–8. https://doi.org/10.1002/cyto.b.21722 PMID: 30328261

7. De Propris MS, Intoppa S, Milani ML, Mariglia P, Nardacci MG, Peragine N, et al. ROR1 is an accurate and reliable marker of minimal residual disease in chronic lymphocytic leukaemia. British Journal of Haematology. 2020; 190(6):e394–e9. https://doi.org/10.1111/bjh.16910 PMID: 32579248

8. Atakan-Ozturk HB, Falay M, Albayrak M, Yildiz A, Ozturk CP, Maral S, et al. CD81 Expression in the Differential Diagnosis of Chronic Lymphocytic Leukemia. Clin Lab. 2019; 65(3). Available from: https://doi.org/10.7754/Clin.Lab.2016.180802 PMID: 3068852

9. Sorigue M, Juncà J, Sarrate E, Grau J. Expression of CD43 in chronic lymphoproliferative leukemias. Cytometry B Clin Cytom. 2018; 94(1):136–42. https://doi.org/10.1002/cyto.b.21509 PMID: 28073173

10. Sandes AF, de Lourdes Chauvaille M, Oliveira CR, Maekawa Y, Tashiro N, Takao TT, et al. CD200 has an important role in the differential diagnosis of mature B-cell neoplasms by multiparameter flow cytometry. Cytometry B Clin Cytom. 2014; 86(2):98–105. https://doi.org/10.1002/cyto.b.21128 PMID: 24243815

11. Wright GJ, Jones M, Puklavec MJ, Brown MH, Barclay AN. The unusual distribution of the neuronal/lymphoid cell surface CD200 (OX2) glycoprotein is conserved in humans. Immunology. 2001; 102 (2):173–8. https://doi.org/10.1046/j.1365-2567.2001.01163.x PMID: 11260322

12. Debord C, Robillard N, Theisen O, Godmer P, Graveleau J, et al. CD200 expression in flow cytometry helps to distinguish mantle cell lymphoma from other CDS-positive B-cell neoplasms. Haematol Oncol. 2018; 36(3):807–9. https://doi.org/10.1002/hon.2511 PMID: 29656538

13. Palumbo GA, Patriniello N, Fargione G, Cardillo K, Chiarenza A, Berretta S, et al. CD200 expression may help in differential diagnosis between mantle cell lymphoma and B-cell chronic lymphocytic leukaemia. Leuk Res. 2008; 33(9):1212–6. https://doi.org/10.1016/j.leukres.2009.01.017 PMID: 19230971

14. Sewardow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016; 127(20):2375–90. https://doi.org/10.1182/blood-2016-01-643568 PMID: 26980727

15. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood. 2018; 131(25):2745–60. https://doi.org/10.1182/blood-2017-09-806398 PMID: 29540348

16. Starostka D, Kriegova E, Kudelka M, Mikula P, Zehnalova S, Radvansky M, et al. Quantitative assessment of informative immunophenotypic markers increases the diagnostic value of immunophenotyping in mature CD5-positive B-cell neoplasms. Cytometry Part B: Clinical Cytometry. 2018; 94(4):576–87. https://doi.org/10.1002/cyto.b.21607 PMID: 29220870

17. D’Arena G, Vitale C, Rossi G, Coscia M, Ormedè P, D’Auria F, et al. CD200 included in a 4-marker modified Matutes score provides optimal sensitivity and specificity for the diagnosis of chronic lymphocytic leukaemia. Hematol Oncol. 2018; 36(3):543–6.

18. Fan L, Miao Y, Wu YJ, Wang Y, Guo R, Wang L, et al. Expression patterns of CD200 and CD148 in leukemic B-cell chronic lymphoproliferative disorders and their potential value in differential diagnosis. Leuk Lymphoma. 2015; 56(12):3329–35. https://doi.org/10.3109/10428194.2015.1030642 PMID: 25791119

19. Anderson KC, Bates MP, Slaughenhoupt BL, Pinkus GS, Schlossman SF, Nadler LM. Expression of human B cell-associated antigens on leukemias and lymphomas: a model of human B cell differentiation. Blood. 1984; 63(6):1424–33. PMID: 6609729
20. F EA, AA O, E. AEH. Expression and diagnostic utility of single and combined CD200, CD148 and CD160 markers in mature B cell neoplasms as revealed by ROC and SVM analyses. World Academy of Sciences Journal. 2019(1):136–44.

21. Köhnke T, Wittmann VK, Büchlein VL, Lichtenegger F, Pasalic Z, Hiddemann W, et al. Diagnosis of CLL revisited: increased specificity by a modified five-marker scoring system including CD200. Br J Haematol. 2017; 179(3):480–7. https://doi.org/10.1111/bjh.14901 PMID: 28832948

22. Gill KZ. Mantle cell lymphoma mimicking chronic lymphocytic leukemia/small lymphocytic lymphoma on flow cytometry. Journal of Hematopathology. 2020; 13(1):25–31.

23. Mongeau-Marceau Z, Cohen S, Mollica L, LeBlanc R, Fortin B, Terra R, et al. Atypical Chronic Lymphocytic Leukemia with CD200 Expression Shares Similar Outcomes to Classical Chronic Lymphocytic Leukemia. Blood. 2016; 128(22):5572. Available from: http://doi.org/10.1182/blood.V128.22.5572.5572.

24. Sorigue M, Magnano L, Miljkovic MD, Nieto-Moragas J, Santos-Gomez M, Villamor N, et al. Positive predictive value of CD200 positivity in the differential diagnosis of chronic lymphocytic leukemia. Cytometry B Clin Cytom. 2020; 98(5):441–8. https://doi.org/10.1002/cyto.b.21849 PMID: 31692239

25. van Dongen JJ, Lhermitte L, Böttcher S, Almeida J, van der Velden VH, Flores-Montero J, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia. 2012; 26(9):1908–75. https://doi.org/10.1038/leu.2012.120 PMID: 22552007

26. Spacek M, Karban J, Radek M, Babunkova E, Kvasnicka J, Jaksa R, et al. CD200 Expression Improves Differential Diagnosis Between Chronic Lymphocytic Leukemia and Mantle Cell Lymphoma. Blood. 2014; 124(21):5637. Available from http://doi.org/10.1182/blood.V124.21.5637.5637.

27. Sun Y, Wang SA, Sun T. Composite mantle cell lymphoma and chronic lymphocytic leukemia/small lymphocytic lymphoma. Cytometry B Clin Cytom. 2018; 94(1):148–50. https://doi.org/10.1002/cyto.b.21512 PMID: 28109040

28. Dorfman DM, Shahsafaei A. CD200 (OX-2 membrane glycoprotein) expression in b cell-derived neoplasms. Am J Clin Pathol. 2010; 134(5):726–33. https://doi.org/10.1309/AJCP38XRRUGSQQVC PMID: 20959655

29. Lesesve JF, Tardy S, Frotscher B, Latger-Cannard V, Feugier P, De Carvalho Bittencourt M. Combination of CD160 and CD200 as a useful tool for differential diagnosis between chronic lymphocytic leukemia and other mature B-cell neoplasms. Int J Lab Hematol. 2015; 37(4):486–94. https://doi.org/10.1111/ijlh.12315 PMID: 25470765

30. Challagundla P, Medeiros LJ, Kanagal-Shamanna R, Miranda RN, Jorgensen JL. Differential expression of CD200 in B-cell neoplasms by flow cytometry can assist in diagnosis, subclassification, and bone marrow staging. Am J Clin Pathol. 2014; 142(6):837–44. https://doi.org/10.1309/ AJCPBV9ELXC0ECVL PMID: 25389338

31. Rahman K, Kumar P, Gupta R, Singh MK, Nityanand S. Role of CD200 in differential diagnosis of mature B-cell neoplasm. Int J Lab Hematol. 2017; 39(4):384–91. https://doi.org/10.1111/ijlh.12637 PMID: 28422443

32. Hoffmann J, Rother M, Kaiser U, Thrun MC, Wilhelm C, Gruen A, et al. Determination of CD43 and CD200 surface expression improves accuracy of B-cell lymphoma immunophenotyping. Cytometry B Clin Cytom. 2020; 98(6):476–82. https://doi.org/10.1002/cyto.b.21936 PMID: 32716606