Original Article

The potential differential diagnosis value and clinical significance of CD35 expression in B-chronic lymphoproliferative disorders

Yu Shi[#], Lu Liu[#], Hui Yang[#], Xiao Chen, Yan Wang, Sishu Zhao, Huimin Jin, Yujie Wu

Department of Hematology, The First Affiliated Hospital of Nanjing Medical University (Jiangsu Provincial People's Hospital), Nanjing, China

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[#]These authors contributed equally to this work.

Correspondence to: Yujie Wu. Department of Hematology, The First Affiliated Hospital of Nanjing Medical University (Jiangsu Provincial People's Hospital), Nanjing 210029, China. Email: wuyujie123456@163.com.

Background: Flow cytometry for immunophenotyping is the main method for diagnosing chronic lymphocytic leukemia (CLL). Differential diagnosis between CLL and other B-chronic lymphoproliferative disorders (B-CLPDs) is sometimes difficult. This study aimed to investigate whether cluster of differentiation 35 (CD35) could be a useful marker for the differential diagnosis of CLL and other B-CLPDs.

Methods: The CD35 expression on lymphoma cells from 516 B-CLPD patients (347 CLL, 169 other B-CLPDs) was investigated through flow cytometry analysis. Serum C3 and C4 levels in B-CLPD patients were also evaluated.

Results: The results showed that the expression percentage and mean fluorescence intensity of CD35 were reduced in CLL cases compared with other B-CLPD patients. Furthermore, CD35 <17% produced a sensitivity of 81.8% and a specificity of 88.4% for supporting the diagnosis of CLL. Additionally, the addition of CD35 to Matutes score improved the score's discriminative power. The sensitivity of the Matutes score was improved from 81.3% to 88.5%, and the accuracy was improved from 96.6% to 97.6%. Finally, 15.0% and 16.4% of CLL patients had defective serum C3 and C4 levels at diagnosis, respectively.

Conclusions: Evaluating CD35 expression could have potential differential diagnostic value in distinguishing CLL from other B-CLPDs, especially between CLL and mantle cell lymphoma (MCL).

Keywords: CD35; flow cytometry; chronic lymphocytic leukemia (CLL); differential diagnosis; B-chronic lymphoproliferative disorders (B-CLPDs)

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Introduction

Chronic lymphocytic leukemia (CLL) is a subtype of B-chronic lymphoproliferative disorders (B-CLPDs) and has specific immunophenotypic properties. It mainly occurs in older adults and is characterized by the accumulation of malignant cluster of differentiation 5-positive ($CD5^+$) B lymphocytes in the blood, bone marrow, spleen, and lymphoid organs (1-3). The definitive diagnosis of CLL is mainly based on the following 3 criteria: (I) peripheral blood monoclonal B lymphocyte count reaching $\geq 5\times10^9$/L; (II) morphologic features of a marked increase in small and mature lymphocytes from peripheral smear; and a (III) typical immunophenotype of $CD19^+$, $CD5^+$, $CD23^+$, $CD200^+$, weak CD22, CD79b (dim), $FMC7^-$, and weak surface immunoglobulin (sIg) expression (dim) (4). Multiparameter flow cytometry for immunophenotyping is the main method for the differential diagnosis between CLL and other B-CLPDs. The flow cytometry score, developed by Estella Matutes, is widely used, and CLL typically

displays a score of 4 or 5 (5-7). However, some CLL cases may have an atypical immunophenotype with a Matutes score of less than 4, which usually results in diagnostic difficulties. In the leukemic phase, CLL and other B-CLPDs, especially $CD5^+$ B-cell neoplasms, which are mainly mantle cell lymphoma (MCL), can share some similar features. However, their clinical and prognostic features are significantly different (8-11). Therefore, the addition of novel immunophenotypic marker(s) that can help identify B-CLPDs may have considerable clinical value.

CD35, also known as complement receptor 1 (CR1), is a cell surface glycoprotein distributed on the surface of a variety of cells, including red blood cells, granulocytes, monocytes, mast cells, follicular dendritic cells, glomerular foot process cells, B cells and some CD4+ T cells. The high- and low-affinity ligands of CD35 are C3b/C4b and C3bi/C3c, respectively (12-14). It has been reported that CD35 is also a receptor for C1q, but the role of CD35 as a C1q receptor remains to be further studied (15). CD35 has an immune adhesion function, and can mediate the combination of immune complexes and red blood cells, monocytes, neutrophils, macrophages, B lymphocytes, or eosinophils in a covalent form. CD35 can also accelerate the degradation of the C3 and C5 convertases, further regulating complement system activation. In addition, CD35 serves as a cofactor, participating in the limited cleavage of C4b and C3b, with the resulting fragments being the ligands of other complement receptors (16,17). A previous study reported that the expression of CD35 on B CLL cells was reduced or even negative in patients with CLL (18). This suggests that investigations into the addition of CD35 expression in the differential diagnosis of B-CLPDs could help to reach a definitive conclusion which can distinguish CLL from other B-CLPDs.

In this study, the significance of CD35 expression in leukemic B cells of patients with CLL and other B-CLPDs was investigated. The results confirmed that the surface expression of CD35 on leukemic B cells was reduced or even negative in patients with CLL compared with other B-CLPDs. The cut-off value of CD35 in the differential diagnosis of CLL was also determined and the addition of CD35 to Matutes score improved the score's discriminative power. Consequently, CD35 has been comprehensively proved to be a useful marker for the differential diagnosis between CLL and other mature B-cell neoplasms, especially between CLL and MCL. We present the following article in accordance with the STARD reporting checklist (available at https://dx.doi.org/10.21037/atm-21-3199).

Methods

B-CLPD characteristics

In the research, peripheral blood (PB) or bone marrow (BM) samples collected from patients diagnosed with B-CLPDs from January 2015 to February 2020 were examined. According to the World Health Organization (WHO) 2016 classification of Tumors of Haematopoietic and Lymphoid Tissues and its revisions, consecutive B-CLPD patients were further diagnosed as CLL, MCL, follicular lymphoma (FL), waldenstrom macroglobulinemia (WM), marginal zone lymphoma (MZL), or hairy cell leukemia (HCL) (19). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics board of Jiangsu Province Hospital (No. 2021-SRFA-208) and individual consent for this retrospective analysis was waived.

B-CLPDs are classified according to peripheral blood cell count, morphological analysis of peripheral blood or bone marrow, and immunophenotyping of cells by flow cytometry. CLL is diagnosed by the standard criteria of microscopic cell morphology and flow cytometry analysis with calculation of the Matutes score. For typical CLL cases, the Matutes score is 4 or 5, while atypical CLL cases are identified by a score <4. The expression of cyclin D1, the translocation of *IGH/CCND1* or the presence of t(11;14) is used to either diagnose or exclude MCL, while the presence of t(14;18) is used to diagnose FL. "Hair cells" can be seen in the peripheral blood, bone marrow, liver, or spleen of patients with HCL. The diagnosis of MZL requires histopathological examination of the corresponding tissues.

Immunophenotypic analysis

Fresh PB and BM samples were examined by flow cytometric analysis according to the procedure described below. First, 2 mL of PB or BM was aseptically drawn into anticoagulant tubes containing ethylene diamine tetra-acetic acid (EDTA). Then, 100 μL of PB/BM was taken and washed with phosphate-buffered saline (PBS) to remove the free light chains, after which 500 μL of erythrocyte lysate was added. After 10 minutes, the same amount of PBS was added and mixed. The supernatant was removed 5 minutes later, and then 1 mL of PBS was added for washing. Next, the appropriate monoclonal antibodies (mAbs) were added. After being incubated for 15 minutes at room temperature in darkness, the samples were washed with PBS and

resuspended with 500 μL of PBS. At least 50,000 cells were analyzed by flow cytometer (Beckman Coulter, USA).

The mAbs applied in the study were CD45, CD19, CD5, CD23, CD10, CD22, CD20, CD79b, FMC7, CD103, CD11c, CD25, CD38, CD49d, CD138, CD200 (Immunotech SA, Marseilles, France), and SmIg (Dako, Glostrup, Denmark). Corresponding isotype antibodies were used as a negative control. In addition, CD35 (BD Biosciences, NJ, USA) was tested in all cases.

The lymphocyte population was defined as low forward scatter/low side scatter/CD45^{high}, and subsequent analysis was conducted on CD19$^+$ B cells. Positivity was identified as a ≥30% positive cell population. In general, sIgM and CD22 were counted as a score of 1 when its expression was weak. CD5 and CD23 were counted as a score of 1 when the positive cell population was ≥30%. FMC7 was counted as a score of 1 when the positive cell population was <30%. In addition, the weak, moderate, or high expression pattern was assessed based on the log scale of the fluorescence axis on the B-cell population. The gating strategy involved specifically identifying CD35 on CD19 single-positive or CD5$^+$CD19$^+$ double-positive lymphoma cells, depending on the subtype of B-CLPDs. The CD35 expression percentage and mean fluorescence intensity (MFI) were calculated in lymphoma cells by analysis software after the flow cytometry data were acquired.

Measurement of complement C3 and C4

The blood samples were collected within 24 hours for measuring complement C3 and C4. The concentration of complement C3 and C4 was detected by immune scatter turbidimetry (normal values: C3 0.79–1.52 g/L; C4 0.16–0.38 g/L). Measurements were conducted using the BN2 special protein analyzer and matching reagents (Siemens AG, Munich, Germany), according to the manufacturer's protocol.

Statistical analysis

All statistical analyses were performed by Graphpad Prism 8 (GraphPad Software, San Diego, CA, USA) and SPSS version 22.0 (IBM Corp., Armonk, NY, USA). The comparison of CD35 expression among B-CLPD groups was calculated by the Kruskal-Wallis test. Statistical significance was defined as a P value less than 0.05. A receiver operating characteristic curve (ROC) and area under the ROC curve (AUC) were established to evaluate the diagnostic value of CD35 in differentiating the B-CLPD subtypes.

Results

Biologic and laboratory characteristics

A total of 516 patients diagnosed with B-CLPDs were included in the study. The most common subtype was CLL (67.2%, n=347), followed by MCL (13.4%, n=69), WM (7.2%, n=37), FL (5.8%, n=30), MZL (4.5%, n=23), and HCL (1.9%, n=10). The median age was 62 years old (range, 27–86) at diagnosis. The male:female ratio of B-CLPDs patients was 2.2:1. The male patients were prevalent in the CLL, MCL, MZL and WM subgroups, but there was little difference between males and females in FL and HCL subgroups (*Table 1*).

Anemia and thrombocytopenia were common in B-CLPD patients. Anemia [hemoglobin (HGB) <120 g/L in males; HGB <110 g/L in females] occurred in 38.9% of patients while thrombocytopenia [platelet (PLT) <100×10^9/L] was observed in 30.0% of patients. Anemia occurred in 89.2% of patients with WM, which was the most common subtype. Severe anemia (HGB <60 g/L) was also more likely to occur in WM patients, but rarely occurred in patients with MZL, FL, and CLL (less than 2.6%). Thrombocytopenia most often occurred in WM patients, with an incidence rate of 32.4%. However, only a slight proportion of patients with thrombocytopenia suffered from the severe deficiency associated with the condition. Hyperleukocytosis [white blood cell (WBC) >10×10^9/L] was observed in some patients with CLL (11.0%), while leukopenia (WBC <4×10^9/L) occurred in 45.9% and 50% of WM and HCL patients, respectively. The non-hematological laboratory characteristics, including B symptoms, hepatomegaly, splenomegaly, and lymphadenopathy at diagnosis, are also listed in *Table 1*. Approximately 35.3% of patients had B symptoms. Splenomegaly was commonly apparent in patients with MCL (44.9%) and MZL (39.1%). The incidence of hepatomegaly was not high in any of the B-CLPD subtypes. Lymphadenopathy was more common in patients with CLL (56.5%), MCL (47.8%), and FL (66.7%).

The expression pattern of CD35 in B-CLPDs

CD35 expression was tested in 516 B-CLPD cases. The CD5$^+$CD19$^+$ double-positive lymphoma cells showed weak CD35 expression in CLL patients (*Figure 1*). The

Table 1 The clinical characteristics of patients with B-CLPDs (n=516)

Characteristic	CLL	MCL	FL	MZL	WM	HCL
Median age [range]	62 [27–86]	63 [33–83]	51.5 [35–69]	61 [45–84]	67 [50–82]	55.5 [43–80]
Male/female	2.0/1	3.3/1	0.9/1	4.8/1	3.6/1	1.5/1
WBC ($\times10^9$/L), mean [range]	46.2 [0.7–540.4]	32.3 [1.3–518.0]	14.3 [2.6–80.4]	49.4 [2.8–746.1]	4.5 [0.8–9.9]	12.9 [1.6–59.3]
ALC ($\times10^9$/L), mean [range]	39.8 [0.3–470.4]	25.8 [0.6–431]	8.9 [0.1–74.3]	42.2 [0.9–696.1]	1.5 [0.3–5.5]	10.1 [0.7–52.8]
HGB (g/L), mean [range]	118.0 [34–193]	108.0 [38–161]	124.6 [73–160]	112.0 [63–161]	76.9 [40–134]	94.5 [58–127]
PLT ($\times10^9$/L), mean [range]	133.2 [2–451]	137.6 [14–411]	174.5 [78–418]	155.9 [15–426]	153. 9 [15–312]	132.2 [42–259]
B symptom, n (%)	115 (33.1)	34 (49.3)	8 (26.7)	11 (47.8)	12 (32.4)	2 (20.0)
Hepatomegaly, n (%)	14 (4.0)	6 (8.7)	3 (10.0)	1 (4.3)	2 (5.4)	1 (10.0)
Splenomegaly, n (%)	90 (25.9)	31 (44.9)	4 (13.3)	9 (39.1)	7 (18.9)	2 (20.0)
With nodal involved ≥4, n (%)	196 (56.5)	33 (47.8)	20 (66.7)	5 (21.7)	7 (18.9)	2 (20.0)

B-CLPD, B-chronic lymphoproliferative disorder; CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma; FL, follicular lymphoma; MZL, marginal zone lymphoma; WM, waldenstrom macroglobulinemia; HCL, hairy cell leukemia; WBC, white blood cell; ALC, absolute lymphocyte count; HGB, hemoglobin; PLT, platelet.

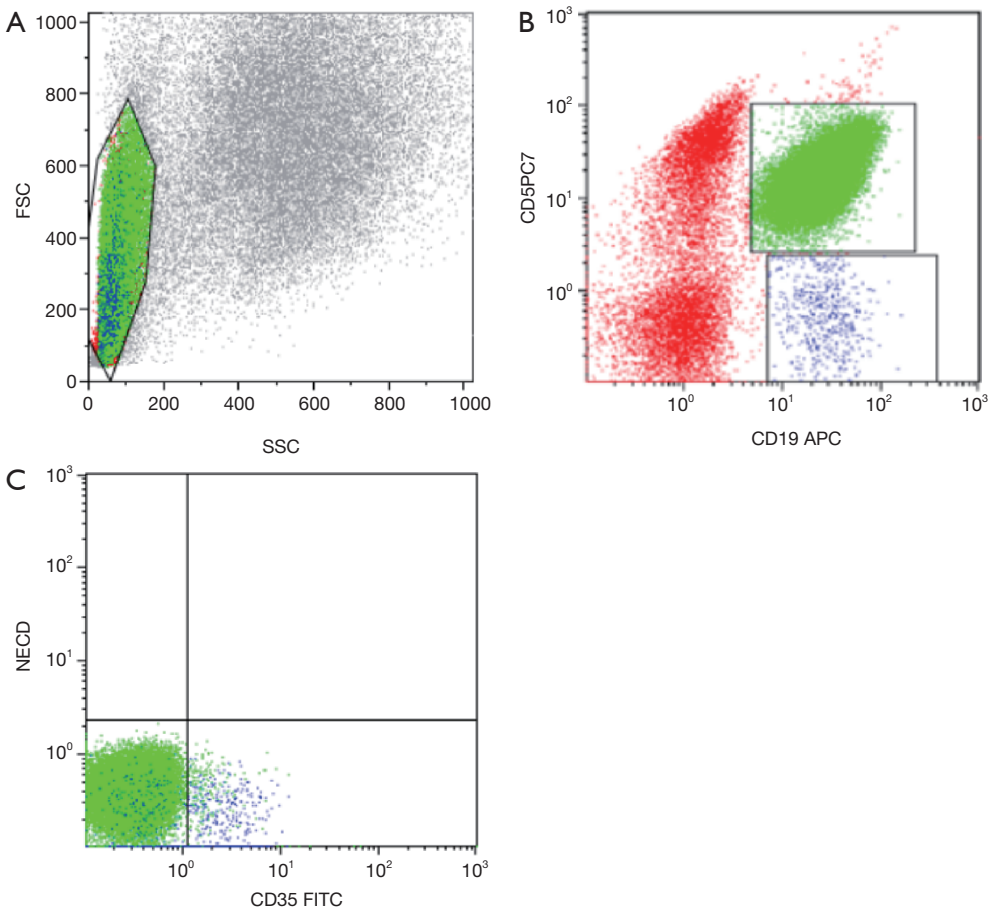

Figure 1 Flow cytometry measurements of B cells or lymphoma cells in a representative case of CLL using multiple antibodies. The low forward scatter/low side scatter/CD19+/CD5+ lymphoma cells of this patient with CLL show weak CD35 expression compared to low forward scatter/low side scatter/CD19+/CD5- B cells. CLL, chronic lymphocytic leukemia; CD, cluster of differentiation.

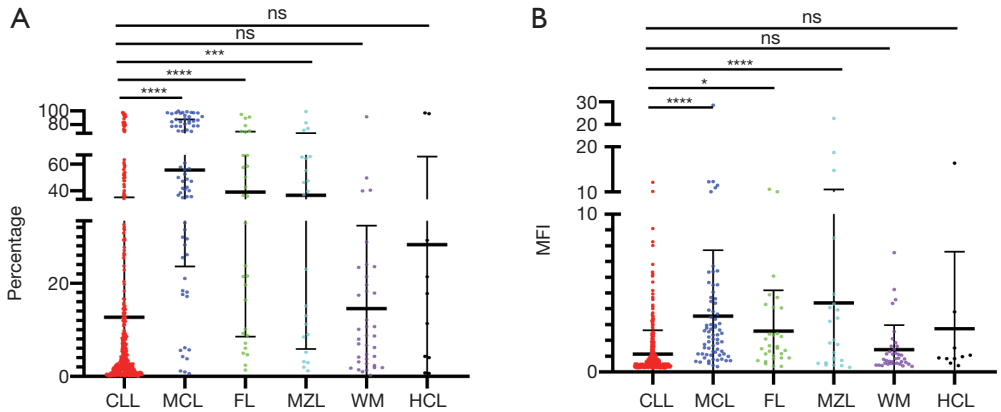

Figure 2 The distribution of CD35 expression in B-CLPDs. (A) The expression percentage of CD35 on leukemic B cells in B-CLPDs. (B) CD35 MFI of leukemic B cells for B-CLPDs. Error bars: mean ± SEM. *P<0.05, ***P<0.001, and ****P<0.0001; ns: no significant difference between the two groups. CD, cluster of differentiation; B-CLPDs; B-chronic lymphoproliferative disorders; MFI, mean fluorescence intensity.

Table 2 The MFI and expression percentage of CD35 in B-CLPD patients

Variable	CLL	MCL	FL	MZL	WM	HCL
MFI						
Mean	1.13	3.54	2.59	4.37	1.41	2.73
Median	0.54	2.36	1.56	1.83	0.83	0.93
Interquartile ranges						
Min	0.24	0.34	0.35	0.29	0.36	0.4
P25	0.38	1.14	0.92	0.66	0.55	0.84
P50	0.54	2.36	1.56	1.83	0.83	0.93
P75	1.08	4.36	3.66	4.19	1.45	1.41
Max	12.1	28.49	10.6	22.68	7.57	16.4
Percentage (%)						
Mean	12.7	55.5	38.8	36.5	14.6	28.2
Median	2.89	55.5	34.31	36.92	8.66	14.56
Interquartile ranges						
Min	0.03	0.61	1.38	1.21	0.23	0.63
P25	0.98	29.85	9.46	8.74	3.11	4.12
P50	2.89	55.5	34.31	36.92	8.66	14.56
P75	11.51	84.89	66.40	64.64	19.64	27.24
Max	97.58	99.77	94.93	99.16	91.35	96.95

MFI, mean fluorescence intensity; B-CLPD, B-chronic lymphoproliferative disorder; CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma; FL, follicular lymphoma; MZL, marginal zone lymphoma; WM, waldenstrom macroglobulinemia; HCL, hairy cell leukemia.

expression percentage and mean fluorescence intensities of CD35 were studied in B-CLPD patients. The distribution characteristics of CD35 in B-CLPD patients are shown in *Figure 2*. The mean MFI of CD35 in CLL, MCL, FL, MZL, WM, and HCL was 1.13 (range, 0.24–12.1), 3.54 (range, 0.34–28.49), 2.59 (range, 0.35–10.6), 4.37 (range, 0.29–22.68), 1.41 (range, 0.36–7.57), and 2.73 (range, 0.4–16.4), respectively. Meanwhile, the mean expression percentage of CD35 in CLL, MCL, FL, MZL, WM, and HCL was 12.7% (range, 0.03–97.58%), 55.5% (range, 0.61–99.77%), 38.8% (range, 1.38–94.93%), 36.5% (range, 1.21–99.16%), 14.6% (range, 0.23–91.35%), and 28.2% (range, 0.63–96.95%) respectively. The median and interquartile ranges of CD35 MFI and expression percentage in CLL, MCL, FL, MZL, WM, and HCL are also shown in *Table 2*. CD35 MFI and expression percentage of CLL were lower than those of MCL, FL, and MZL, and the differences were statistically significant. Although the CD35 MFI and expression percentage of CLL were also lower than those of WM and HCL, there were no significant differences between these groups. Many membrane markers have been used in B-CLPDs that can clearly distinguish between CLL, FL, MZL and HCL. However, CLL and MCL are both CD5-positive B-CLPDs, and it is sometimes difficult to distinguish CLL from MCL using morphology and immunophenotyping. Significant differences were observed in the MFI and expression percentage of CD35 (P<0.0001) between CLL and MCL, suggesting that these 2 diseases could be differentiated by combining CD35 with effective markers (CD5, CD23, FMC7, CD22, CD79b, sIg).

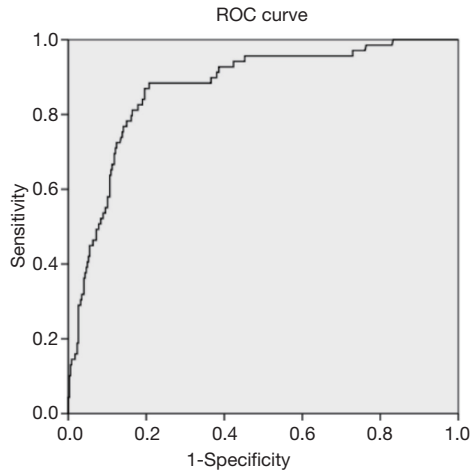

Figure 3 Receiver operating characteristic curve of CD35 expression to predict the diagnostic value of CD35 in differentiating CLL and MCL. A strong separation between these two subgroups in CD35 expression was reflected with an AUC of 0.873. AUC, area under the curve; CD, cluster of differentiation; CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma.

Table 3 χ^2 analysis of CD35 expression in patients with CLL and MCL

CD35	CLL (n=347)	MCL (n=69)	Total (n=416)	P value†
Positive	63	61	124	P<0.001
Negative	284	8	292	
Total	347	69	416	

†Significance tested is P<0.05. CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma.

Table 4 Patients classification according to Matutes score and the alternative proposed score

MS	Matutes score		MS with CD35 added	
	CLL	MCL	CLL	MCL
≥5	117	0	253	0
4	165	0	54	4
3	39	8	30	4
2	23	27	8	31
1	3	34	2	30
0	0	0	0	0

CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma.

The differential diagnostic value of CD35 for CLL and MCL

ROC curve analysis was used to determine the predictive value of CD35 for CLL and MCL. A strong separation between these two subgroups was evident according to an ROC plot with an AUC of 0.873 [95% confidence interval (CI): 0.828–0.918] for CD35 (*Figure 3*). CD35 <17% had a sensitivity of 81.8% and a specificity of 88.4% for diagnosing CLL. CD35 expression was <17% in 284/347 of CLL cases, while it was ≥17% in 61 of 69 MCL cases. A statistically significant difference (P<0.001) in CD35 expression was observed between CLL and MCL patients (*Table 3*). CD35 <17% supported the diagnosis of CLL. In other words, CD35 was considered score 1 when the positive cell population was <17%. Of the 347 CLL cases in this study, 282 CLL cases had the typical CLL phenotype (Matutes score ≥4), while 65 cases showed an atypical phenotype (Matutes score <4). The Matutes score of all patients with MCL was less than 4. With the addition of CD35 to Matutes score, the Matutes score of 25 patients with atypical CLL changed from 3 to 4, while 4 cases in MCL group had a modified MS of 4 (*Table 4*). When adding CD35 to CD5, CD23, CD22, FMC7 and sIgM, the sensitivity and accuracy of the MS improved from 81.3% to 88.5%, and from 96.6% to 97.6%, respectively, and a high specificity was maintained (P<0.05; *Table 5*).

The C3 and C4 serum complement levels in B-CLPDs

Several reports have shown the complement system in CLL patients to be deficient. Therefore, the C3 and C4 serum complement levels in 516 cases of B-CLPDs were evaluated: 15.0% (52/347) of CLL patients had a defective serum C3 level, while 21.9% (37/169) of other B-CLPD patients had a defective serum C3 level at diagnosis. No significant difference was found between CLL and other B-CLPDs in C3 serum complement level deficiency (P=0.051; *Table 6*). Similar results were also found in C4 component serum levels between CLL and other B-CLPDs (*Table 7*). Furthermore, 57 CLL cases had a defective serum C4 level while 38 other B-CLPD patients had a defective serum C4 level at diagnosis. In conclusion, a correlation was not found between C3 or C4 serum complement level deficiency and B-CLPD subtypes (P>0.05).

Discussion

Immunophenotyping by flow cytometry is a common

Table 5 Sensitivity, specificity and accuracy of Matutes scoring systems in differential diagnosis between CLL and MCL

Scoring system	Sensitivity % (95% CI)	Specificity % (95% CI)	CLL *vs.* MCL % (95% CI)
CD5, CD23, FMC7, SmIg, CD22	81.3 (76.8–85.2)	100.0 (94.8–100.0)	96.6 (94.4–98.1)
CD5, CD23, FMC7, SmIg, CD22, CD35	88.5 (84.6–91.6)	94.2 (85.8–98.4)	97.6 (95.6–98.8)

CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma.

Table 6 χ^2 analysis of C3 component serum levels in patients with CLL and other B-CLPDs

C3	CLL (n=347)	Other B-CLPDs (n=169)	Total (n=516)	P value†
Normal	295	132	427	P=0.051
Deficiency	52	37	89	
Total	347	169	516	

†Significance tested is P<0.05. CLL, chronic lymphocytic leukemia; B-CLPD, B-chronic lymphoproliferative disorder.

Table 7 χ^2 analysis of C4 component serum levels in patients with CLL and other B-CLPDs

C4	CLL (n=347)	Other B-CLPDs (n=169)	Total (n=516)	P value†
Normal	290	131	421	P=0.096
Deficiency	57	38	95	
Total	347	169	516	

†Significance tested is P<0.05. CLL, chronic lymphocytic leukemia; B-CLPD, B-chronic lymphoproliferative disorder.

and effective method for distinguishing CLL from other B-CLPDs. The diagnosis of CLL is definite when characteristic immunophenotypic features ($CD5^+$, $CD19^+$, $CD23^+$, $CD22^{-/dim}$, $CD79b^{-/dim}$, $FMC7^-$, sIg^{dim} and $CD20^{dim}$) exist (4). However, making a differential diagnosis of CLL from other B-CLPDs is difficult in the absence of typical immunophenotypic features. The distinction between CLL, FL, MZL, and HCL can be made via membrane markers or different morphological characteristics. CLL and MCL are sometimes not easily distinguished by morphology and immunophenotyping. Some atypical phenotypes of CLL can be expressed as CD23 negativity, bright expression of CD20 or CD22, and FMC7 positivity (20,21). Although CLL and MCL may share many similar morphologic and immunophenotyping features, the clinical evolution and therapeutic regimens of these two B-CLPD subtypes are divergent. Due to the difficulty in differentially diagnosing CLL and MCL, considerable research has been devoted to finding convenient and effective markers that might be used to distinguish CLL from MCL, such as CD18, CD54, and CD148 (22,23). Additional tests such as immunohistochemistry (IHC) for cyclin D1 or SOX11 and fluorescence in situ hybridization (FISH) analysis of t(11;14) have been used to differentiate CLL from MCL.

CD35 is a complement receptor which can be expressed on B lymphocytes and play an important role in the regulation of humoral immunity (14,24). Several studies have indicated that the expression of CD35 is significantly lower in patients with CLL. However, the sample capacity of these articles was small and did not compare the expression of CD35 in various B-CLPDs (18).

This study investigated the expression of CD35 on B leukemic cells of patients with CLL and other B-cell neoplasms by flow cytometry. The expression of CD35 was presented as an expression percentage, and MFI was calculated by analysis software. The results indicated that CD35 was expressed in B-CLPDs with significantly different expression levels. A high expression of CD35 was observed in MCL, FL, and MZL cases. CD35 expression of patients with WM and HCL was moderate, while CD35 had the lowest expression in CLL patients. The differences in CD35 expression between CLL and MCL, FL, MZL groups were statistically significant, while the differences between CLL and WM, HCL groups were not significant. The expression of CD35 was significantly different between CLL and other B-CLPDs which suggested that CD35 may be a useful marker in the differential diagnosis of CLL. Determining the predictive value of CD35 for CLL and MCL was the focus of this study. Subsequently, ROC curve analyses were conducted to assess CD35 expression for the diagnosis of CLL. The results showed that the sensitivity and specificity were up to 81.8% and 88.4% respectively at the cut-off value of 17%. The classical Matutes score was effective in separating CLL from MCL when the Matutes score was high (≥4), and 65 cases with atypical CLL overlapped with MCL at a Matutes score ≤3. With

the addition of CD35 to the Matutes score, 25 cases were reclassified into classical CLL and distinguished from MCL. Indeed, the alternative proposed score in this study achieved a high accuracy in CLL diagnosis.

It has been reported that CLL patients have a high incidence of an impaired complement system and low serum complement level. CLL patients are related to autoimmune features and poor prognosis (25,26). Therefore, this study measured the C3 and C4 serum complement levels in 516 B-CLPD cases. It was found that 15.0% (52/347) and 16.4% (57/347) of CLL patients, respectively, had defective serum C3 and C4 levels, but there were no significant differences between CLL and other B-CLPDs in C3 and C4 serum complement level deficiency. Complement deficiencies in CLL patients are a critical clinical issue and have implications for monoclonal antibody (mAb) therapy. Complement deficiencies lead to the attenuated induction of complement-dependent cytotoxicity (CDC) and can reduce clinical efficacy of mAb therapy in CLL patients. Supplement of fresh-frozen plasma (FFP) in parallel with mAb therapy can enhance the clinical efficacy of the mAb therapy via the restoration of complement levels to their normal state (27). Although many reports consider the supplement of FFP as beneficial to the treatment of CLL patients, supplying FFP has not been widely adopted in clinical trials and the treatment effect has not been as good as expected (28-30). CD35, a major cellular receptor for C3b/C4b, is essential for regulating complement system activation (13,31). We thus speculate that ineffective FFP supplement therapy may be related to the lack of CD35 expression in CLL patients. In addition, CLL patients often have autoimmune system diseases, including common autoimmune cytopenias (AIC) and relatively less frequent autoimmune diseases, such as autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), pure red cell aplasia (PRCA), Sjögren syndrome and AID-related arthritis (32). Reduced CD35 expression was also observed in patients with AIHA, Sjögren syndrome, paroxysmal nocturnal hemoglobinuria (PNH), and systemic lupus erythematosus (SLE) (33). Consequently, whether CLL patients being easily complicated with autoimmune diseases is associated with a deficiency of CD35 expression needs to be further studied.

At the time of diagnosis, patients with CLL should undergo risk stratification according to the CLL International Prognostic Index (CLL-IPI). Patients who have the low- and intermediate-risk CLL-IPI group should be monitored every 6–12 months, while those who have the high- and very high-risk CLL-IPI group should be provided with treatment in early period or monitored every 3–6 months. Previously, patients were mainly treated with standard chemoimmunotherapy regimens. In recent years, bruton's tyrosine kinase (BTK) inhibitors, phosphatidylinositol-3-kinase (PI3K) inhibitors, and B cell lymphoma-2 (BCL-2) inhibitors make a breakthrough in CLL treatment. Chimeric antigen receptor T-cell (CAR-T) therapy has also begun to show a certain effect. In our study, CD35 is only used as a diagnostic indicator. Whether there is a difference in treatment between $CD35^+$ CLL and $CD35^-$ CLL needs to be further studied.

In conclusion, CD35 seems to be a useful additional marker for routine laboratory analysis in the diagnosis of ambiguous CLL cases. Furthermore, CD35 detection by flow cytometry analysis is convenient and inexpensive when peripheral blood and/or bone marrow samples are available. Based on our findings, we recommend adding CD35 to the B-CLPD flow cytometric routine panels in CLL diagnosis.

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Footnote

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