Original Article

Flowcytometry Aiding Morphological Diagnosis of Mature B-cell Neoplasm in Patients with Lymphocytosis

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Abstract

Background: Mature B-cell neoplasms (MBCNs) are a category of disorders with a broad range of clinical manifestations, pathologic features, and outcomes that share common characteristics. They originate from the B-cell lineage, are clonal, and have the immunophenotypic and genetic features of mature B-cells. Flow cytometry (FC) is a widely used method for diagnosing suspected lymphoproliferative disorders in patients with lymphocytosis, lymphadenopathy, and other lymphoproliferative disorders. **Aim:** To evaluate the role of FC in confirming the provisional morphologic diagnosis in patients with MBCNs. **Materials and Methods:** This is a retrospective; descriptive study conducted on 193 adult patients newly diagnosed with MBCNs; immunophenotypic findings of the patients were reviewed. The B lymphocytes were identified according to their Side-Scattered/CD19 distribution. **Results:** Chronic lymphocytic leukemia (CLL) was the most common subtype (127, 65.9%). Splenomegaly was noted more frequently in hairy cell leukemia variant cases (85.7%) and hairy cell leukemia cases (75%). The anemia at presentation was the least frequent in CLL (20%). The CD5+/CD23+ phenotype of CLL was seen in 114 cases (90%); negativity for FMC7 showed high sensitivity (93.7%) and sufficient specificity (60%) in the diagnosis of CLL. **Conclusions:** It is difficult to diagnose B-chronic lymphoproliferative disorders solely based on morphologic findings. Consequently, FC findings combined with clinical, hematologic, and morphologic features can confidently result in a precise diagnosis.

Keywords: Chronic lymphocytic leukemia, chronic lymphoproliferative disorders, flow cytometry, lymphocytosis

Introduction

Chronic lymphoproliferative disorders (CLPDs) are a diverse group of leukemia and/or lymphomas characterized by the proliferation of mature B lymphoid cells in the peripheral blood (PB), bone marrow (BM), lymph nodes/spleen, and other lymphoid tissue.[1] B-cell neoplasms tend to mimic stages of natural B-cell differentiation, so they can be classified according to the normal stage to some degree. Nevertheless, some common B-cell neoplasms such as hairy cell leukemia do not appear to follow the typical B-cell differentiation level. Lineage heterogeneity or even more rarely, lineage plasticity can be found in some neoplasms. As a result, the neoplastic cell's natural counterpart cannot be used as the sole basis for classification.[2] Because it is difficult to diagnose hematologic disorders solely based on morphologic observations, so to make a reliable histologic diagnosis, alternative diagnostic modalities such as phenotyping via flow cytometry (FC) and immunohistochemistry are needed.[3]

FC immunophenotyping is an essential part of a diagnostic method to deal with hematologic neoplasms; the results should always be interpreted in conjunction with a complete blood cell count, cytomorphologic assessment of blood, BM smears, and BM biopsy, as well as cytogenetic and molecular findings (if indicated).[4] FC is one of the most common and indispensable instruments for the identification, classification, and prediction of malignant hematologic disease.[5] FC is a popular method for diagnosing CLPDs in patients who have

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lymphadenopathy, lymphocytosis, or other symptoms that may indicate a lymphoproliferative disorder (LPD); its studies not only support a malignant diagnosis but also allow for accurate categorization into distinct pathologies. The detection of unusual CD5 expression on a B-cell LPD is extremely helpful in the diagnostic process because it significantly narrows the differential considerably.[6] Even if flow analyses are unable to reliably categorize a B-cell LPD, as is the case with CD5−ve and CD10−ve B-cell disorders, the identification of a monoclonal B-cell indicates the presence of a suspected malignant condition and results in further patient care.[7]

Numerous antigens that are expressed absolutely on B-cells have been recognized, including CD19, CD22, CD79a, CD79b, and other components of the B-cell receptor complex. The CD19 is the most widely used flow cytometric B-cell gating marker, though it can be found at all stages of a B-cell's life cycle, from early progenitors to terminally differentiated plasma cells.[8] The CD20 expression is more limited, starting late in the development of immature B-cells, persisting in mature B-cells, and decreasing during plasma cell differentiation; its expression can be used to distinguish between mature and immature B-cell processes because of its timing and strength of expression.[9]

Materials and Methods

A retrospective, descriptive study was conducted on 193 newly diagnosed adult patients with mature B-cell neoplasms (MBCNs) from January 2014 to October 2016. The study was based on retrieving the data from the archives of the Flow Cytometry Laboratory at the Bone Marrow Transplant Center in Baghdad, Iraq. The diagnosis of MBCNs was based on clinical features, peripheral and/or BM lymphocytes morphology, and immunophenotypic analysis. Clinical information, such as age, gender, clinical presentation, and physical examination (presence of peripheral lymphadenopathy, hepatomegaly, and splenomegaly), was extracted from clinical records of all patients. Hematologic data collected included total white blood cell (WBC) count, absolute lymphocyte count (ALC), percentage of lymphocytes, hemoglobin level, and platelet count.

Inclusion criteria include:

- Newly diagnosed patients with MBCNs,
- Adult patients (>15 years),
- Patients with absolute lymphocytosis (>3.0 × 10^9/l).

The specimens were collected and prepared for morphologic analysis using Bain BJ and Lewis SM's published techniques.[10] The BM aspirate smears and PB specimens were collected and air-dried with Leishman's stain before being analyzed under a light microscope for red blood cell (RBC) count, WBC, and platelet morphology as well as the presence of atypical lymphocytes. Anticoagulant ethylenediaminetetraacetic acid tube was used to collect PB or BM samples. The samples were analyzed directly or stored in a refrigerator (2–8°C), if necessary, and all specimens were analyzed within 24 h of collection. Four-color flow cytometric analysis was performed using a BD FACS Calibur flow cytometer (Becton Dickinson, Bio) and FACSCanto II flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). To ensure that each cell population is accurately defined, 10,000 events were measured per tube. CellQuest software (Becton Dickinson Immunocytometry Systems) and FACSDiva software were used to analyze the data.

Statistical Analysis

SPSS24 (Statistical Packages for Social Sciences, Version 24.0; IBM Corp, Armonk, NY, USA) was used to analyze the results. The data were presented in simple measures of frequency, percentage, median, and range (minimum–maximum values). The positive predictive value (PPV) is the probability of disease presence based on a positive test result, whereas the negative predictive value (NPV) is the probability of disease absence in the presence of a negative test result. The sensitivity, specificity, PPV, and NPV were tested using Conditional Probability Equations.[11,12]

Results

This study included a total of 193 patients with MBCNs; there were 124 men and 69 women with a male:female (M:F) ratio of 1.8:1. The median age was 60 years with a range of 20–90 years. CLL was the most common subtype (127, 65.9%), followed by splenic marginal zone lymphoma (SMZL) and mantle cell lymphoma. There were 127 patients with CLL: 80 (63%) men and 47 (37%) women (M:F ratio 1.7:1) with a median age of 61 years (range 20–90 years). SMZL was observed in 32 patients: 19 (59.4%) men and 13 (40.6%) women (M:F ratio 1.5:1) with a median age of 60 years (range 32–80 years). The mantle cell lymphoma (MCL) was diagnosed in 12 patients: 11 (91.7%) men and 1 (8.3%) woman with an M:F ratio of 11:1, a median age of 64 years (range 42–85 years). The remaining patients' demographic information is summarized in Table 1.

Pallor was the most common in diffuse large B-cell lymphoma (DLBCL), where the frequent percentage was 57.1% followed by MCL (41.7%) and CLL (30%). Fever was most commonly noted in half of the hairy cell leukemia (HCL) cases, followed by MCL (33.3%). Lymphadenopathy was found in 58.3% of MCL and 50% of CLL followed by 40.6% of SMZL cases. Splenomegaly was noted in 85.7% of hairy cell leukemia variant (HCL-v) cases, 75% of HCL cases, and 71.4% of DLBCL cases, whereas hepatomegaly was most frequent in HCL-v (57.1%). Other diseases (lymphoplasmacytic lymphoma [LPL], follicular lymphoma [FL], and prolymphocytic

leukemia [PLL]) showed 100% different clinical findings, but they were inconclusive due to small sample size. The relevant clinical presentations for 193 patients at the time of diagnosis are shown in Figure 1.

Based on hematologic parameters, median hemoglobin concentration was around 9 g/dl in all diseases except in CLL and LPL, which was 11.6 and 7.8, respectively. The anemia (Hb < 10 g/dl) at presentation was the least frequent in CLL (about 20%), whereas it was the most frequent in MCL (83.3%). The median WBC count was the highest in MCL (79.5 × 109/l), whereas it was the lowest in HCL (5.5 × 10^9/l). The median of ALC for both HCL and DLBCL was the lowest among all other diseases, which was 3.9 × 109/l and 5.1 × 109/l, respectively. The median of lymphocytes percentage was around 70% in CLL, SMZL, HCL, HCL-v, and PLL; 81.2% in MCL; and 43.1% in DLBCL. Thrombocytopenia was the most frequent finding in HCL (75%), followed by HCL-v (71.4%) and DLBCL (71.4%); however, the lowest incidence was observed in CLL, which was 25.2% of cases, as shown in Figure 2.

In this study, HCL had the most frequent morphologic true diagnosis, about 75% (3/4) of HCL cases compatible with morphologic diagnosis, followed by CLL, among which 70.9% (90/127) of cases were compatible with morphologic diagnosis. About 58% of MCL and 43% of SMZL were misdiagnosed morphologically as having CLL, whereas about 57% of DLBCL were misdiagnosed as having acute leukemia. The morphologic diagnosis of HCL had both high PPV (85.7%) and NPV (97.3%); the MCL and SMZL had a very low PPV of 20% and 0% for both, respectively, as shown in Table 2.The expression patterns of CD5/CD23 and CD5/CD10 were studied in relation to various subtypes of mature B-cell non-Hodgkin lymphoma. Cases were broadly grouped into four categories based on the combination of antigenic expression of CD5/CD23 and CD5/CD10. The PPV and NPV of these antigenic expression patterns were then calculated. The CD5+/CD23+ antigenic expression pattern is most frequently seen in CLL cases (89.8% of CLL cases), with high sensitivity (89.8%) and specificity (72.7%) for the diagnosis of CLL. Other cases showing such patterns include SMZL (40.6% of cases) and MCL (25% of cases) but with a low PPV of 21.1% for SMZL and 2.3% for MCL. The CD5−/CD23-antigenic expression pattern was seen in all cases of HCL-v and in 75% of HCL, whereas it was seen in only 21.9% of SMZL cases; this pattern had 100% of NPV but a low PPV 31.6% of HCL-v, as shown in Table 3.

The CD5+/CD10-antigenic expression pattern was seen in all cases of MCL and in the vast majority of CLL cases (94.5%), with other cases showing such patterns, including SMZL, in 53.1% of cases. The PPV was highest in CLL

Table 1: Demographic summary of patients

	Sex		M:F (ratio)	Mean of age (years)	Median of age (years)	Range of age (years)
	Male	Female				
CLL (*n* = 127)	80 (63.0%)	47 (37.0%)	1.7:1	61.3	61	20–90
SMZL (*n* = 32)	19 (59.4%)	13 (40.6%)	1.5:1	59.1	60	32–80
MCL (*n* = 12)	11 (91.7%)	1 (8.3%)	11:1	64	65	42–85
HCL (*n* = 4)	2 (50.0%)	2 (50.0%)	1:1	51	46.5	41–70
HCL–V (*n* = 7)	4 (57.1%)	3 (42.9%)	1.3:1	54.3	45	31–83
DLBCL (*n* = 7)	5 (71.4%)	2 (28.6%)	2.5:1	57.4	59	37–70
LPL (*n* = 2)	2 (100.0%)	0	2:0	62	62	59–64
PLL (*n* = 1)	0	1 (100.0%)	0:1	71	NA	71
FL (*n* = 1)	1 (100.0%)	0	1:0	48	NA	48

CLL: chronic lymphocytic leukemia; DLBCL: diffuse large B-cell lymphoma; FL: follicular lymphoma; HCL: hairy cell leukemia; HCL-v: hairy cell leukemia variant; LPL: lymphoplasmacytic lymphoma; MCL: mantle cell lymphoma, PLL: prolymphocytic leukemia, SMZL: splenic marginal zone lymphoma

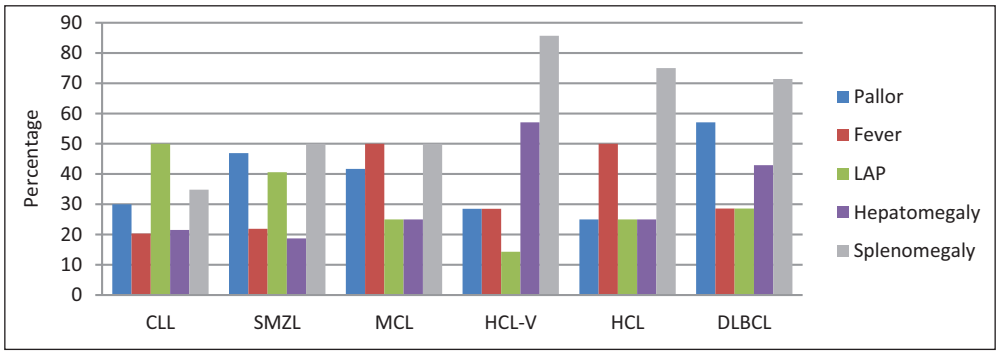

Figure 1: Clinical features of patients with selected mature B-cell neoplasm at diagnosis

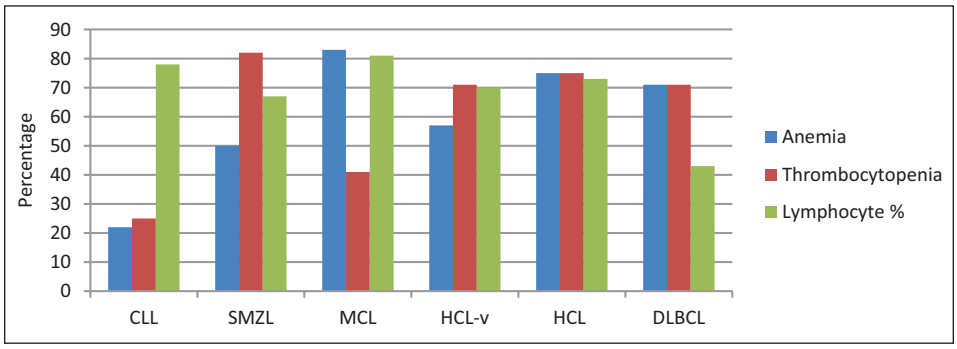

Figure 2: Hematologic parameters of patients with selected mature B-cell neoplasm at diagnosis

Table 2: The diagnostic predictive value of the morphology

	PPV (%)	NPV (%)
CLL	84.9	57.5
SMZL	0.0	83.3
MCL	20	94.1
HCL	85.7	97.3

CLL: chronic lymphocytic leukemia; HCL: hairy cell leukemia; NPV, negative predictive value; PPV, positive predictive value; MCL: mantle cell lymphoma; SMZI: Splenic marginal zone lymphoma

(78.9%) whereas it was lowest in SMZL (11.2%). The CD5−/CD10-antigenic expression pattern was seen in all cases of HCL and HCL-v; it was also seen in 46.9% (15 cases) of SMZL cases and 4.8% (6 cases) of CLL cases. This pattern had a low PPV for all cases but a high NPV for all cases, apart from CLL cases, which had 24.3% of NPV, as shown in Table 4.

Discussion

In the present study, CLL remained the most common B-CLPD (65.9%); this result is in accordance with Oberley *et al.* who reported a frequency of 66%.[13] The median age at diagnosis was 61 years in patients with CLL and it was similar to that reported by Hasan *et al.*[14] and Teke *et al.*[15] On the contrary, the median age at diagnosis in Western countries is much higher, ranging from 67 to 72 years old.[16-18] In terms of clinical presentation, the incidence of splenomegaly was less frequent, whereas lymphadenopathy and hepatomegaly were very close to that reported by Hasan *et al.*[14] These clinical presentations were less frequent than other studies.[16,19] The diversity of clinical presentations in CLL and correlations with ethnicity have been well documented in previous research.[20] The incidence of anemia and thrombocytopenia in the current study was in agreement with Swerdlow *et al.*,[21] whereas it is less frequent than what was reported by Hasan *et al.*[14]

By morphology alone, in about 71% of the PB or BM smears the likely diagnosis is CLL with accepted PPV of 84.9% and low NPV of 57.5%. The low NPV is due to the high number of CLL cases being falsely diagnosed as non-CLL on a morphologic basis. These findings are in agreement with Zhang *et al.*, who reported that a presumptive diagnosis of CLL can be made based on morphology alone in 75.2% of the PB or BM smears.[22] The CD5+/CD23+ phenotype, which is pathognomonic of CLL, was seen in 114 cases (90%) and had a PPV of 88.3% and NPV of 79.2%. This result is very close to what was reported by Gujral *et al.*,[23] who had demonstrated that the phenotype of CD5+/CD23+ for the diagnosis of SLL/CLL was highly specific and had a high PPV (98%) and NPV (73%), and this was observed in only 90% of the cases. Further, CD5+/CD23+ had a high sensitivity (89.8%) and specificity (72.7%) for the diagnosis of CLL and this is in accordance with Köhnke *et al.*[24] Negativity for FMC7 shows high sensitivity and sufficient specificity (93.7% and 60%, respectively) in the differential diagnosis of CLL; these results were comparable with the study by Köhnke *et al.*,[24] who reported the high sensitivity and specificity of negative FMC7 (90.4% and 74.4%, respectively). The lower specificity value in our study was due to the high number of negative FMC7 in non-CLL cases. The CLL cases showed other expression patterns of CD5/CD23, that is, 4.6% showed the CD5+/CD23− phenotype; these cases were labeled as atypical CLL. The frequency of CD23 negativity in CLL is very low: in some patients, it may be difficult to distinguish between CLL and MCL on FC and these cases might need an LN biopsy for confirmation. When we analyzed the expression of SmIg, CD11c, FMC7, and CD79b to find out which of these markers help to differentiate CD23 negative CLL from MCL, it was observed that moderate to bright expression of SmIg in MCL helped to differentiate MCL from CD23 negative CLL. In this study, 50% of MCL cases have moderate to bright expression of SmIg and was dimly expressed in approximately 17% (1/6) of CD23 negative CLL, in agreement with Gujral *et al.*[23] From our perspective, CD11c is an important immunologic marker for distinguishing MCL from aCLL, even though its expression was dim or heterogeneous. CD11c was expressed in 66% of atypical CLL cases, and it was not expressed in MCL cases. CD79b was moderately expressed in 100% of our MCL cases, whereas it was dimly expressed in 50% of atypical CLL cases. FMC7 was expressed in 55.6% of MCL cases and in 16.7% of aCLL, those results were in accordance with Starostka *et al.*[25]

These findings emphasize the diagnostic value of CD79b, FMC7, CD11c, and SmIg in the differentiation of MCL from atypical CLL.

In this study, about 7% of CLL (CD5+/CD23+) cases expressed heterogeneous to dim monoclonal kappa light chains, with 15% expressing dim to moderate monoclonal lambda light chains. None of the aCLL (CD5+/CD23−) cases had monoclonal kappa light chains, whereas monoclonal lambda light chains were heterogeneously expressed in 16.7%. Twenty-two percent of MCL cases expressed monoclonal kappa light chains and 66.7% expressed monoclonal lambda light chains, which is lower than that reported by Hasan *et al.*[14] (67.6% and 32.4%, respectively) and Starostka *et al.*[25] (50% and 39.4%, respectively), mostly due to technical issues.

SMZL, according to the findings of this study, is a disease of middle-aged patients, with a median age of 60 years, which is consistent with the findings of many other studies.[26] SMZL seems to affect males more than females; gender prevalence is controversial (46), but there is an increasing trend of male predominance.[27] Splenomegaly and anemia are the most common clinical signs and they were observed in 50% of patients; however, thrombocytopenia has been reported in 25.2% of cases. These results are in agreement with Piris *et al.*[27] and Behdad *et al.*[28] According to our results, the morphology had no diagnostic value, as Zhang *et al.* found that BM core biopsy and/or immunophenotyping data are needed in combination with PB morphology to support the diagnosis.[22] The CD5 was expressed in 53.1% of cases and this frequency is slightly higher than that of Al-Anizi *et al.*,[26] who reported an overall incidence of CD5 expression in 41% of cases, and in contrast with Santos *et al.*,[29] who reported an overall incidence of CD5 positivity in 10%–25% of the cases. Ortolani[30] observed CD5 positivity in 12%–50% of SMZL cases. Another report by Jevremovic *et al.*[31] raises the possibility that the variable expression of CD5 on SMZL can be attributed to the variation in the detection techniques and the microenvironment. NPV for CD5−/CD23− and CD5−/CD23+ phenotypes to detect SMZL was 84.7% and 85.6%, respectively, which is in accordance with Gujral *et al.*[23]; however, the PPV for CD5−/CD23−, CD5−/CD23+ was low (36.8% and 53.3%, respectively). This was most likely due to the high number of CD5−/CD23− and CD5−/CD23+ in our non-SMZL cohort. CD23 was expressed in 66% and CD11c was expressed in 54.8%; these results are in accordance with Santos *et al.*[29] and very close to what was reported by Al-Anizi *et al.*,[26] who found CD23 and CD11c positivity in 59% and 50%

Table 3: Expression pattern and diagnostic performance of CD5/CD23 in various mature B-cell non-Hodgkin lymphomas

	CLL (n = 127)		SMZL (n = 32)		MCL (n = 12)		HCL (n = 4)		HCL-v (n = 6)*	
	N %	PPV NPV	N %	PPV NPV	N %	PPV NPV	N %	PPV NPV	N %	PPV NPV
CD5+ and CD23+	114 89.8	88.3% 79.2%	13 40.6	10.1% 64.1%	3 25	2.3% 83.0%	0 0	0.0% 92.5%	0 0	0.0% 88.7%
CD5+ and CD23−	6 4.7	26.3% 27.0%	4 12.5	21.1% 82.8%	9 75	47.4% 98.2%	0 0	0.0% 98.1%	0 0	0.0% 96.3%
CD5− and CD23+	7 5.5	40.0% 29.3%	8 25	53.3% 85.6%	0 0	0.0% 92.8%	1 25	6.6% 98.2%	0 0	0.0% 96.4%
CD5− and CD23−	0 0	0.0% 23.9%	7 21	36.8% 84.7%	0 0	0.0% 92.6%	3 75	15.8% 99.3%	6 100	31.6% 100%

CLL: chronic lymphocytic leukemia; HCL: hairy cell leukemia; HCL-v: hairy cell leukemia variant; NPV, negative predictive value; PPV, positive predictive value; MCL: mantle cell lymphoma; SMZI: Splenic marginal zone lymphoma

*The total number of HCL-v cases was seven but one case had CD5−ve and CD23 was not tested

Table 4: Expression pattern and diagnostic performance of CD5/CD10 in various mature B-cell non-Hodgkin lymphomas

	CLL (n = 127)		SMZL (n = 32)		MCL (n = 12)		HCL (n = 4)		HCL-v (n = 7)	
	N %	PPV NPV	N %	PPV NPV	N %	PPV NPV	N %	PPV NPV	N %	PPV NPV
CD5+ and CD10+	0 0	0.0% 35.7%	0 0	0.0% 83.4%	0 0	0.0% 94.3%	0 0	0.0% 97.9%	0 0	0.0% 96.4%
CD5+ and CD10−	120 94.5	78.9% 85.4%	17 53.1	11.2% 63.4%	12 100	7.9% 100%	0 0	0.0% 90.2%	0 0	0.0% 82.9%
CD5− and CD10+	0 0	0.0% 34.3%	0 0	0.0% 83.1%	0 0	0.0% 93.6%	0 0	0.0% 97.9%	0 0	0.0% 96.3%
CD5− and CD10−	7 5.5	16.2% 24.3%	15 46.9	40.5% 89.1%	0 0	0.0% 92.9%	4 100	10.8% 100%	7 100	23.3% 100%

CLL: chronic lymphocytic leukemia; HCL: hairy cell leukemia; HCL-v: hairy cell leukemia variant; NPV, negative predictive value; PPV, positive predictive value; MCL: mantle cell lymphoma; SMZI: Splenic marginal zone lymphoma

of SMZL cases, respectively. CD103 and CD123 were negative in all cases, which was in agreement with Behdad *et al.*[28] Regarding CD25 expression, we noted a positivity for this HCL marker in 4% (1/26) of cases, which was in accordance with Al-Anizi *et al.*[26] (3%); on the other hand, Starostka *et al.*[25] observed a higher figure (54%). CD25 was negative in 96% of cases, so it readily differentiated SMZL cases from HCL cases; there was only one positive case for CD25 with heterogeneous expression and one negative case for both CD103 and CD123 that resembled HCL-v but with negativity for CD11c, which favored the diagnosis of SMZL. These results are in accordance with Behdad *et al.*,[28] who reported that SMZL virtually never exhibits strong expression of CD103, CD11c, and CD25.

The median age of patients with MCL at diagnosis was 64 years, with a striking male predominance (92%) with an M:F ratio of 11:1. Splenomegaly and lymphadenopathy were the most common clinical features seen in 58.3% of cases, in agreement with that reported by McKay *et al.*[32] In correlation with morphology, only one case (8.3%) was truly diagnosed as MCL, which is in accordance with what was found by Zhang *et al.*, where one case (16.7%) was likely to be diagnosed as MCL.[22] The CD5+/CD23− antigenic expression pattern, which is classical of MCL,[23] was expressed in 75% (9/12) of MCL cases, with a high NPV of 98%. Absence of this pattern ruled out the diagnosis of MCL, except for three cases; these results are in accordance with Gujral *et al*.,[23] who found a high NPV of 99% for this pattern. Three cases of MCL have the CD5+/CD23+ phenotype (which is classical for CLL) but with positive CD79b, and FMC7 with bright CD20 and CD22 expression whereas it is negative for light chain restriction, which favored the diagnosis of MCL. These results are in accordance with Morice *et al.*[33] who had shown that CD22 and CD20 expression could help to differentiate CLL from MCL or other non-CLL cases, with both, CD22 and CD20, showing dim expression in CLL and bright expression in non-CLL cases.

When analyzing HCL and HCL-v, four cases of HCL cases were reviewed. The median age at diagnosis was 47 years, which is in accordance with Khorshid *et al.*[34] (47 years); on the other hand, Somasundaram *et al*.[35] observed a slightly higher figure (50 years). The M:F ratio was 1:1 and this was in disagreement with Khorshid *et al.*[34] and Somasundaram *et al*.,[35] who reported a more frequent occurrence in males; it may be related to the relatively smaller number of cases studied compared with other series or may be due to ethnical variation. In the present study, seven HCL-v cases were included. The median age at diagnosis was 45 years with a slight male predominance; these results are in agreement with Swerdlow *et al.*,[21] who stated that HCL-v is a disease of the middle age to elderly and there is a slight male predominance. The most recent Western studies show that HCL-v is a disease of the elderly with a median age of 70 years.[36] However, CD103 has the highest specificity for HCL and HCL-v, and it is rarely expressed in other B-cell lymphoproliferative disorders.[37] A previous study showed that all cases of HCL were positive for CD103,[36] whereas CD103 expression in HCL-v was variable ranging from greater than half to 100% of cases.[36,37] CD103 was reliably expressed in all HCL cases (100%) and in about two-third of HCL-v cases (71.4%), and this is consistent with Matutes *et al.*[37] Generally, SMZL was negative for CD103 and we found that HCL and HCL-v are clearly distinguished from SMZL by CD103; all of our SMZL cases were CD103 negative. HCL is known for its bright CD25 expression, whereas HCL-v is known for its lack of CD25 expression. Angelova *et al.*[36] reported infrequent expression of CD25 in HCL-v cases (14% and was usually dim/partial). However, in our series, CD25 was reliably positive in all HCL and negative in HCL-v, and this is in accordance with Shao *et al.*[38] and Matutes *et al.*[37] We found that CD25 could reliably differentiate HCL from HCL-v (75% sensitivity and 100% specificity). HCL-v without both CD25 and CD103 may be difficult to differentiate from SMZL.[39] We notably identified that two cases of HCL-v were negative for both markers, but with bright CD11c expression, which favored HCL-v over SMZL, and it is comparable with Shao *et al.*[38] and Venkataraman *et al.*[40]

Due to the small sample size, the results of these disorders (DLBCL, LPL, PLL, and FL) were inadequate for comparison with other studies.

Conclusions

FC immunophenotyping can be considered as an important and highly sensitive tool for the evaluation of B-cell neoplasms, and it has the highest diagnostic efficacy in the confirmation of B-CLPD. Immunophenotyping by FC can be useful in distinguishing MCL from aCLL as well as SMZL from HCL and HCL-v. The use of CD103 and CD25 in a standard panel is valuable for determining the diagnosis of HCL and HCL-v. The present study identified that CD103 can be specific for HCL and HCL-v. It is difficult to diagnose B-CLPD solely based on morphologic findings. Consequently, FC findings combined with clinical, hematological, and morphologic features can result in a precise diagnosis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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