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A COMPARISON OF BONE MARROW FLOW CYTOMETRY FINDINGS AND BONE MARROW BIOPSY İMMUNOHISTOCHEMICAL FINDINGS IN CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA: A METHODOLOGICAL STUDY

KRONİK LENFOSİTİK LÖSEMİ/KÜÇÜK LENFOSİTİK LENFOMADA KEMİK İLİĞİ AKIM SITOMETRİSİ BULGULARI İLE KEMİK İLİĞİ BIYOPSİSİ İMMÜNOHİSTOKİMYASAL BULGULARININ KARŞILAŞTIRILMASI: METODOLOJİK BİR ÇALIŞMA

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ABSTRACT

Introduction: The diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), which also includes atypical forms, can be very difficult. The aim of this study is to compare immunohistochemical (IHC) and flow cytometric (FC) immunophenotyping methods in the CLL/SLL, the most common mature B-cell leukemia/lymphoma of adults, in bone marrow biopsy. In these two methods, it is to determine the minimum panels that can differentiate CLL/SLL from other mature B-cell leukemia/lymphomas.

Material and Method: The study included 68 adult patients diagnosed with mature B cell leukemia/lymphoma. Final diagnoses were made on the basis of peripheral smear, bone marrow aspirate, bone marrow biopsy, IHC and FC analysis, clinical and genetic studies. Cell counts, final diagnosis and immunophenotyping were compared with both methods. Cytogenic findings were evaluated. The sensitivity, specificity, positive and negative predictive values of IHC and FC results for CLL/SLL were calculated.

Results: CLL/SLL was diagnosed in 64.7% of the cases and non-CLL/SLL in 35.29% of the cases. The median age was 66.50 years, 69.18% male and 30.82% female. The diagnosis compatibility in FC and IHC was found to be highest in hairy cell leukemia/lymphoma with 100%, and in CLL/SLL with 90%. In other lymphomas, a definitive diagnosis could only be made with biopsy. Immunophenotyping made with CD23+CD43+CD5+LEF-1 in the bone marrow biopsy showed a sensitivity of 95.45% (range, 84.53-99.44%) and a specificity of 100.00% (range, 85.75-100.00%) for CLL/SLL. Immunophenotyping made with CD23+CD43+CD5+CD200+ in FC showed a sensitivity of 100.00% (range, 91.96-100.00%) and a specificity of 82.61% (range, 61.22-95.05%) for CLL/SLL.

Conclusion: This was the most extensive study to have compared immunophenotyping in IHC and FC in bone marrow biopsies of mature B cell leukemia/lymphoma. The addition of LEF-1 to the CD23(+), CD43(+), CD5(+) panel in IHC and the addition of CD200 to the same panel in FC will be extremely helpful in the differential diagnosis of typical/atypical CLL/SLL from mature B cell leukemia/lymphoma.

Keywords: Lymphoma, Immunohistochemistry, Bone Marrow, Flow Cytometry

ÖZET

Giriş: Atipik formları da olan kronik lenfositik lösemi/küçük lenfositik lenfomanın (KLL/SLL) teşhisi çok zor olabilir. Bu çalışmanın amacı erişkinlerde en sık görülen matür B hücreli lösemi/lenfoma olan KLL/SLL'de kemik iliği biyopsisinde immünohistokimyasal (İHK) ve akım sitometrik (AS) immünfenotipleme yöntemlerini karşılaştırmaktır. Bu iki yöntemde KLL/SLL'yi diğer matür B hücreli lösemi/lenfomalardan ayırt edebilecek minimum panelleri belirlemektir.

Yöntem: Çalışmaya matür B hücreli lösemi/lenfoma tanılı 68 erişkin hasta dahil edildi. Kesin tanı periferik yayma, kemik iliği aspirasyonu, kemik iliği biyopsisi, İHK analizi ve AS analizi, klinik ve genetik çalışmalar sonucunda konuldu. Hücre sayıları, kesin tanı ve immünofenotipleme her iki yöntemle karşılaştırıldı. Sitogenik bulgular değerlendirildi. KLL/SLL için İHK ve AS sonuçlarının duyarlılığı, özgüllüğü, pozitif öngörü ve negatif öngörü değerleri hesaplandı.

Bulgular: Olguların %64,7'sinde KLL/SLL, %35,29'unda KLL/SLL dışı lenfomalar saptandı. Ortanca yaş 66,5 olup, %69,18'i erkek ve %30,82'si kadındı. AS ve İHK'da tanı uyumluluğu %100 ile en yüksek tüylü hücreli lösemi/lenfomada oldu. KLL/SLL'de %90 olarak bulundu. Diğer lenfomalarda kesin tanı ancak biyopsi ile konulabildi. Kemik iliği biyopsisinde CD23+CD43+CD5+LEF-1 ile yapılan immünofenotipleme, KLL/SLL için %95,45 duyarlılık (aralık, %84,53-99,44) ve %100,00 özgüllük (aralık, %85,75-100,00) gösterdi. AS'de CD23+CD43+CD5+CD200+ ile yapılan immünofenotipleme, KLL/SLL için %100,00 duyarlılık (aralık, %91,96-100,00) ve %82,61 özgüllük (aralık, %61,22 -95,05) gösterdi.

Sonuç: Çalışmamız matür B hücreli lösemi/lenfomanın kemik iliği biyopsisinde İHK ve AS'de immünofenotiplemeyi karşılaştıran en kapsamlı çalışmadır. İHK'da CD23(+), CD43(+), CD5(+) paneline LEF-1'in, AS'de aynı panele CD200'ün eklenmesi klasik tip ve atipik KLL/SLL tanısında ve diğer matür B hücreli lösemi/lenfomalardan ayırıcı tanısında oldukça faydalıdır.

Anahtar kelimeler: Lenfoma, İmmünohistokimya, Kemik İliği, Akım Sitometri

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INTRODUCTION

Mature B cell leukemia/lymphoma, within the group of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), is one of the most frequently seen hematological neoplasms in adults. The World Health Organization (WHO) classification of mature B-cell leukaemia/lymphoma, as in other hematological malignancies, morphology, immunophenotypic characteristics, genetic and clinical findings are used (1). Immunophenotypic classification plays an important role in determining the cell origin of mature B-cell leukemia/lymphoma, as well as in patient management and follow-up, and is a sensitive method (2). Immunophenotyping is performed by immunohistochemical (IHC) studies in bone marrow biopsies and with by flow cytometry (FC) in bone marrow aspirate suspensions (1). These methods are generally used as complementary to each other in the diagnosis, and each has advantages over the other according to the lymphoma subgroup (3). There is a limited number of studies in the literature which have compared these two methods. The aim of this study was to compare these two methods in bone marrow biopsies in adult patients with mature B cell leukemia/lymphoma in CLL/SLL and other mature B cell leukemia/lymphoma, and to determine the most appropriate panel for CLL/SLL diagnosis with these two methods.

MATERIAL AND METHOD

The methodological study included 68 adult patients diagnosed with mature B cell leukemia/lymphoma who underwent bone marrow biopsy and flow cytometry studies between 2018 and 2021.The definitive diagnoses were made as a result of peripheral smear bone marrow aspirate, bone marrow biopsy, flow cytometry cytometric analysis, clinical, radiological, and genetic studies. Classification was made according to the latest WHO revision. The procedures applied to the bone marrow biopsy were as follows: following fixation in 10% formaldehyde and decalcification processes, the tissue was processed routinely and embedded in paraffin. Slices 4 microns in thickness were cut for hematoxylin and eosin staining. Slices 2-3 microns in thickness were cut and placed on polylysine-coated glass slides for IHC studies. An IHC panel formed from CD20, CD5, CD23, CD43, CD19, CD10, Bcl-2, Bcl-6, Sox-11, Cyclin D1, LEF-1, CD22, CD79A, CD25, CD103, annexin, kappa, and lambda antibodies was applied to all the bone marrow biopsy samples [Table 1].

IHC was performed using the antigen retrieval technique and the avidin-biotin-peroxidase complex method. Antibodies were detected on a Leica Bandmax automated immunohistochemistry instrument. The Bond Polymer Refine Detection Kit (Leica, DS9800) was used for each antibody. The required staining procedure on the data sheet was applied, and appropriate positive and negative controls were used for each antibody. In the evaluation of the leukemia/lymphoma cells of the IHC study, with the exception of LEF-1, the percentage of CD3(-) and CD20(+) cells were determined, taking the lymphoma involvement pattern into consideration (4). Accordingly, the positivity grading was divided into 5 groups of 0:no reaction obtained, 1(+): <5%, 2(+): 5-9%, 3(+): 10-20%, 4(+): ≥20% (5) [Figure 1]. LEF-1 was scored as negative or positive, and if the majority of leukemic cells were positive, it was accepted as positive [Figure 1] (6).

The procedure applied in the FC study was as follows: Bone marrow aspirate samples taken into tubes containing ethylenediaminetetraacetic acid (EDTA) were processed within 24 hours. All bone marrow samples were tested for CD19, CD20, CD5, CD23, CD38, CD10, CD2, CD200, CD7, CD79a, CD34, CD117, CD43, FMC7, CD14, CD33, MPO, CD103, CD25, CD11c kappa and lambda light chain, antibodies. The reading results of the samples obtained after the application and lysing procedure were evaluated. Readings were performed on Beckman Coulter (Miami, USA) Navios Ex instrument using antibodies from the same company. The instrument was calibrated and compensated before reading. First, lymphocytes were marked on the CD45-Side Scatter (SSC) plot. Antibody expression of 20% or more on cells was considered positive. Antibody staining intensities were also measured as weak, medium and bright [Figure 2] (7).

Kappa and lambda light chain expressions were evaluated to evaluate the monoclonality of their cells. Kappa:lambda ratios outside the range of 3:1 and 0.30:1 were considered monoclonal (8).

Statistical Analysis

For statistical analyses, MedCalc (9) and SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc. statistical programs were used. Continuous data were expressed as median, minimum and maximum values, and categorical data as numbers (n) and percentages (%). The Shapiro-Wilk test was used to assess the conformity of continuous data to the normal distribution, and they were found not to be normally distributed. The Mann-Whitney U test was used to compare unpaired groups. The sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive, negative predictive, and accuracy values were calculated of the IHC results (CD23, CD5, CD43, LEF1 and all 4 together (at least 3 positives of CD23, CD5, CD43 and LEF-1) and the flow cytometry results (CD23, CD5, CD43, CD200 and all 4 together (at least 3 positives of CD23, CD5, CD43, and CD200) for CLL/SLL. Cutoff values for the cell counts obtained from biopsy and flow cytometry were determined as a result of Receiver Operating Characteristic (ROC) curve analysis. A

Table 1: The characteristics of the antibodies used in the immunohistochemical study

	Clone	Dilution Rate	Incubation Time	Antigen Revealing	Company
CD20	L26	1:200	40 minutes	ER2	Leica
PAX5	Polyclonal	1:80	40 minutes	ER2	Thermo
CD10	56C6	1:100	40 minutes	ER2	Leica
CD79A	HM47/A9	1:150	40 minutes	ER2	Thermo
CD19	ZR212	1:100	30 minutes	ER2	Zeta
CD22	SP104	1:50	20 minutes	ER1	Zeta
CD5	4CY	1:100	20 minutes	ER2	Leica
CD23	Polyclonal	1:50	30 minutes	ER2	Leica
CD43	Polyclonal	1:40	30 minutes	ER1	Leica
BCL-2	100/D5	1:50	40 minutes	ER2	Thermo
BCL-6	LN22	1:60	40 minutes	ER2	Leica
SOX-11	ZM101	1:100	30 minutes	ER2	Zeta
CYCLİN-D1	P2D11F11	1:30	40 minutes	ER2	Leica
LEF-1	EP310	1:100	30 minutes	ER2	Epitomics
CD25	SP176	1:70	40 minutes	ER1	Zeta
CD103	EP206	1:100	30 minutes	ER1	BioSB
ANNEXİN	BSB-95	1:200	30 minutes	ER2	BioSB
KAPPA	L1C1	1:900	30 minutes	ER1	Thermo
LAMBDA	SHL53	1:200	10 minutes	ER1	Leica

*ER1: Citrat Buffer, pH:6; ER2: EDTA Buffer, pH:9

value of $p<0.05$ was considered as statistically significant.

RESULTS

The 68 cases included in the study were 69.18% male and 30.82% female with a median age of 66.50 years (20-87 years). CLL/SLL was diagnosed in 64.7% of the cases and non-CLL/SLL B cell lymphoma types in 35.29% of the cases. The CLL/SLL group included cases diagnosed with CLL/SLL and atypical CLL/SLL. The B cell lymphoma types other than CLL/SLL were identified in the Table 2.

Of the 40 cases diagnosed with CLL/SLL by IHC, 36 were also diagnosed by FC. In the cases not diagnosed with CLL/SLL by FC, the diagnosis was reported as an increase in clonal B cells [Table 2]. Of the 4 cases diagnosed with atypical CLL/SLL in the IHC, all were diagnosed as CLL/SLL with FC [Table 2].

In the non-CLL/SLL group, 2 of the 8 cases with IHC diagnosis of mantle cell lymphoma and 2 cases diagnosed with hairy cell leukemia, were seen to be compatible with the FC diagnosis. In 1 patient diagnosed with diffuse large B cell lymphoma, the diagnosis was made with IHC study of the biopsy as no cells could be obtained for FC [Table2].

Fluorescence in situ hybridization (FISH) was performed in 27 of the CLL/SLL cases. As a result of the FISH analysis 13q deletion was seen in 13 (48.15%) cases, p53 deletion in 3 (11.12%), 17p deletion in 1 (3.70%), trisomy 12 in 1 (3.70%), NHL (Non-Hodgkin's lymphoma) in 1 (3.70%), and in 8 (29.63%) cases, no abnormal FISH findings were observed. In the 4 cases with atypical CLL/SLL, 13q deletion was seen in 1, t (14;18) trisomy 12 in 2, and normal FISH findings in 1.

To diagnose CLL/SLL within (NHL), the IHC markers of CD23, CD43, CD5, LEF-1 were used, and the diagnostic performance of these evaluated together is shown in table 3.

Table 2: The diagnoses of the cases and comparisons of the IHC and FC findings in diagnosis

Diagnostic	Cases	IHC and FC Agreement	Diagnostic only Defined By IHC
	%* (n)	%(n)	%(n)
CLL/SLL	67.71 (44/68)		
CLL/SLL	58.83 (40/68)	90.00 (36/40)	10.00 (4/10)
Atypical CLL/SLL	5.88 (4/68)	0 (0)	100.00 (4/4)
Non-CLL/SLL	35.29 (24/68)		
Mantle Cell Lymphoma	11.76 (8/68)	25.00 (2/8)	75.00 (6/8)
Diffuse Large B Cell Lymphoma	7.35 (5/68)	0 (0/5)	100.00 (5/5)
Follicular Lymphoma	5.88 (4/68)	0 (0/4)	100.00 (4/4)
Marginal Zone Lymphoma	4.42 (3/68)	0 (0/3)	100.00 (3/3)
Lymphoplasmacytic Lymphoma	2.94 (2/68)	0 (0/2)	100.00 (2/2)
Hairy Cell Leukemia/Lymphoma	2.94 (2/68)	100.00 (2/2)	0 (0/2)

*CLL/SLL: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma. IHC: Immunohistochemistry. FC: Flow Cytometry, * Percentage of column

CD23 was found to have 97.73% sensitivity, 95.83% specificity, 97.56% positive predictive value (PPV), and 21.82 positive likelihood ratio. CD43 was found to have 93.18% sensitivity, 70.83% specificity, 85.42% PPV, and 3.19 positive likelihood ratio. CD5 was found to have 88.64% sensitivity, 70.83% specificity, 84.78% PPV, and 3.04 positive likelihood ratio. The sensitivity and specificity values for LEF-1 were found to be 100%. When CD23, CD43, CD5, and LEF-1 were evaluated together, the diagnostic performance for those with ≥3 positive results were found to be 95.45% sensitivity, 100.00% specificity, 82.69% PPV, and 21.81 positive likelihood ratio [Table 3].

In the FC diagnosis of CLL/SLL, the diagnostic performances of CD23, CD43, CD5, and CD200 antibodies evaluated singly and together are shown in Table 4. CD23 was found to have 97.73% sensitivity, 60.87% specificity, 82.69% PPV, and 2.50 positive likelihood ratio. CD43 was found to have 97.73% sensitivity, 73.91% specificity, 87.76% PPV, and 3.75 positive likelihood ratio. CD5 was found to have 97.73% sensitivity, 65.22% specificity, 84.31% PPV, and 2.81 positive likelihood ratio. CD200 was found to have 97.73% sensitivity, 100.00% specificity, and 100.00% PPV. When CD23, CD43, CD5, and CD200 were evaluated together, the diagnostic performance for those with ≥3 positive results were found to be 100.00% sensitivity, 82.61% specificity, and 91.67% PPV [Table 4].

When surface light chain expression was evaluated with FC in CLL/SLL and non-CLL/SLL cases, it was seen that of 29 cases with Kappa monoclonality, 19 (65.52%) were CLL/SLL and 10 (34.48%) were non-CLL/SLL. The median Kappa FC percentages were determined to be 40% (range, 25-90%) for CLL/SLL cases, and 51.50% (range, 25-90%) for non-CLL/SLL cases. The value of the CLL/SLL group was seen to be lower than that of the non-CLL/SLL group but the difference was not determined to be statistically significant ($p=0.179$) [Figure 3].

Of the 15 cases with Lambda clonality, 11 (73.33%) were CLL/SLL and 4 (26.67%) were non-CLL/SLL. The median Lambda FC percentages were determined to be 81.36% (range, 60-94%) for CLL/SLL cases, and 67.50% (range,

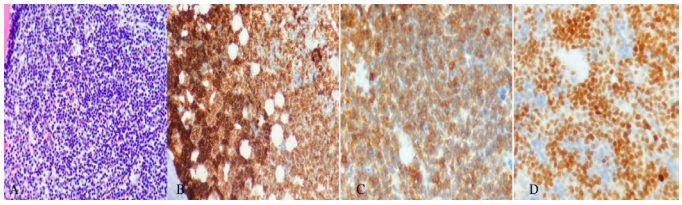

Figure 1. Small-sized atypical lymphocytes that replace bone marrow cells, causing diffuse infiltration, chronic lymphocytic leukemia/small lymphocytic lymphoma (A, x200HE). Strong reaction with CD20 antibody (B, x200), CD5 antibody (C, x200) in chronic lymphocytic leukemia/small lymphocytic lymphoma cell cytoplasm. Strong positive staining with LEF-1 antibody in lymphoma cell nuclei in the same infiltration (D, x200)

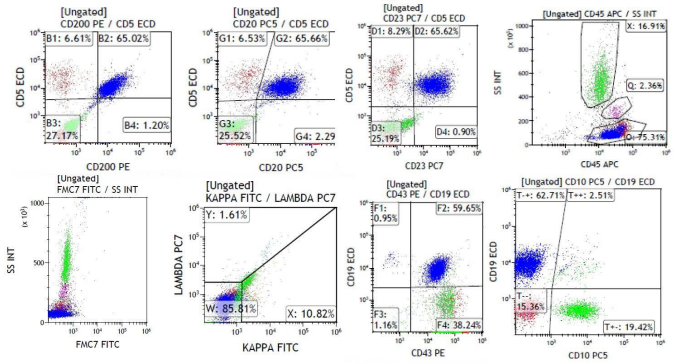

Figure 2. Chronic lymphocytic leukemia/small lymphocytic lymphoma in flow cytometry examination, CD20, CD5, CD200, CD23, CD43, CD19 positivity, negative reaction with kappa, lambda, CD10 and FMC7.

50-75%) for non-CLL/SLL cases. The value of the CLL/SLL group was seen to be statistically significantly higher than that of the non-CLL/SLL group (p=0.010) [Figure 4]. No light chain expression was determined in 24 cases.

In the comparisons of the cell counts obtained immunohistochemically in the bone marrow biopsies of the CLL/SLL and non-CLL/SLL cases, the median cell percentage was determined to be 70% (range, 30-95%) in CLL/SLL cases and 40% (range, 20-80%) in the non-CLL/SLL cases. The cell percentage in the CLL/SLL cases was determined to be statistically significantly higher than that of the non-CLL/SLL cases (p<0.001) [Figure 4].

In the comparisons of the cell counts obtained with FC in the CLL/SLL and non-CLL/SLL cases, the median cell percentage was determined to be 80% (range, 20-98%) in CLL/SLL cases and 36.50% (range, 0-82%) in the non-CLL/SLL cases. The cell percentage in the CLL/SLL cases was determined to be statistically significantly higher than that of the non-CLL/SLL cases (p<0.001) [Figure 4].

As a result of the ROC curve analysis to determine the optimal cutoff value for CLL/SLL cases, when the AUC for the biopsy cell count was 0.779 (95% CI: 0.670-0.888, p<0.001), the cutoff value determined was 55%, which was seen to have 72.70% sensitivity and 62.50% specificity for cell count in bone marrow biopsy. When the AUC of cell count in FC was 0.891 (95% CI:0.809-0.973), the cut-off value determined was 55.50%, which was seen to have 92.20% sensitivity and 79.20% specificity for cell count [Figure 5].

DISCUSSION

Mature B-cell leukemia/lymphoma is common in adults and is usually diagnosed by bone marrow biopsy. Immunophenotyping is an important step in the identification and classification of these diseases. Immunophenotyping can be performed with both IHC and FC in bone marrow biopsies. Of this group of diseases, CLL/SLL is the most common (1).

There are few studies in the literature that have compared these two methods in definitive diagnosis. Comparative studies have been conducted more in pediatric cases with leukemia, and the agreement between FC and IHC in these diseases has been found to be extremely high (10). In previous studies of mature B cell leukemia/lymphoma in adults, the highest agreement of these two methods in diagnosis has been reported to be lymphoma CLL/SLL at 85% (11, 12). In the current study, the agreement of FC and IHC in CLL/SLL was determined to be 90%. The diagnosis of mantle cell lymphoma can be made with IHC studies with cyclin D1 and Sox-11 studies (1). Previous studies have found agreement between FC and IHC in mantle cell lymphoma to be 18% and 48%. In the current study it was 25%. In FL, MZL, DLBCL, and LPL, FC and IHC agreement has been found to be at low levels in previous studies, varying between 12% and 55% (11,12). In the current study, FC and IHC agreement in the infiltration of these lymphomas was determined to be lower than reported in literature. The definitive diagnosis can be made with IHC studies in biopsy. In the current study, the FC and IHC agreement was 100% in hairy cell leukemia, which was higher than the rate of 70% reported in previous studies (11).

Cell loss has been reported in bone marrow biopsies, especially in FC trials. There may be different reasons for this. Many factors may affect the results from the biopsy technique used to the experience of the personnel (13). In the current study, the mean CLL/SLL cell counts were 70% (range, 30-95%) in IHC and 80% (range, 20-98%) in FC. Although the similarity of these mean cell counts was consistent with previous studies, the rates have been reported as 40% and 39% (12), and thus the rates of infiltrating cells in the current study were much higher. A statistically significant difference was determined between the CLL/SLL cases and the non-CLL/SLL cases in respect of the amounts of infiltrating cells in both the FC and IHC studies (p<0.001). As the agreement between the two

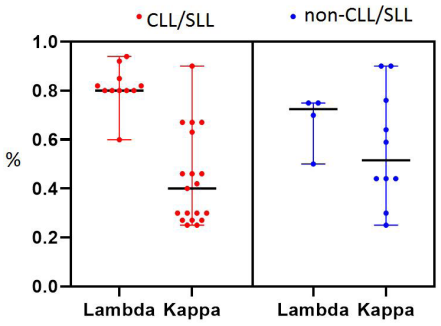

Figure 3. Comparison of the Lambda and Kappa results in biopsy and flow cytometry of the CLL/SLL and non-CLL/SLL cases. Higher incidence of lambda light chain in CLL/SLL when comparing kappa and lambda light chains in terms of clonality in CLL/SLL and other lymphomas.
CLL/SLL: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma. Mann Whitney U Test was used.

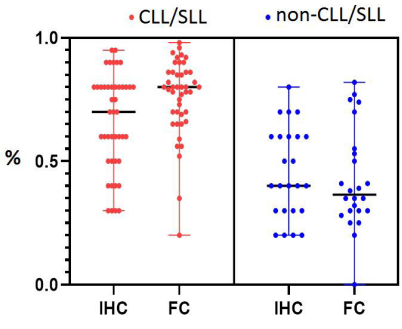

Figure 4. Results of comparisons of cell counts in biopsy and flow cytometry of CLL/SLL and non-CLL/SLL cases. Higher cell counts were obtained in both IHC and FC compared to the other group in CLL/SLL.
IHC: Immunohistochemistry. FC: Flow Cytometry. CLL/SLL: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma.

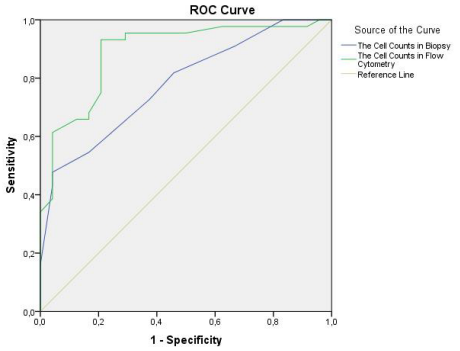

Figure 5. Demonstration of the cut-off value obtained in terms of cell amount, with the ROC curve of high sensitivity and selectivity in detecting CLL/SLL in both methods.
CLL/SLL: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma. (ROC) Receiver Operating Characteristic curve analysis and Mann Whitney U Test were used.

methods was high in the CLL/SLL cases, this suggests that the diagnosis can be confirmed with a single method. Selection of the correct panel is important in both methods for both the diagnosis and for differential diagnosis. Although there are studies in literature with this aim, generally the antibodies used have been examined separately in respect of sensitivity and specificity. In one of these previous studies, CD5 was found to have 100% sensitivity and CD 23, 46% with IHC in bone marrow biopsy in CLL/SLL (5). In the current study CD5 was determined to have 88.64% sensitivity, 70.83% specificity, and 84.78% PPV.
While the sensitivity of CD5 was observed to be relatively low, CD 23 in the current study was found to have 97.73% sensitivity, 95.83% specificity, and 97.56% PPV, which were higher rates than in literature. In the current study CD23 was determined to have high diagnostic value for CLL/SLL in IHC. According to the IHC results, LEF-1 was positive in all the CLL/SLL cases in the current study, which was similar to some previous studies (14). Moreover, the sensitivity and specificity for LEF-1 were 100%, which were higher than reported in literature (6).

With the FC method, CD5 in the current study CLL/SLL cases was found to have 97.73% sensitivity, 65.22% specificity, 84% PPV, and 2.81 positive likelihood ratio. A previous study reported sensitivity of 69% and specificity of 76.90% for CD5 (15). Thus, the sensitivity of CD was higher and specificity was lower in the current study. CD23 in the current study CLL/SLL cases was found to have 97.73% sensitivity, 60.87% specificity, and 82.69% PPV with the FC method. This finding was for CD 23 alone, whereas in previous studies it has generally been calculated together with CD5, with reported sensitivity of 79.80% and specificity of 87.20% (15). Compared with that study the rates of the current study for CD23 alone are higher for sensitivity and lower for specificity.

Currently, CD200 is included by the WHO in the diagnostic criteria for CLL/SLL (16). Immunophenotyping made with CD200 in FC was found to be 100% in all the current study CLL/SLL cases, and previous studies have reported this rate as 99.50% and 100% (17, 18). That CD200 in FC was negative in all the MCLs was consistent with a previous study. CD200 in the current study was found to have 97.73% sensitivity, 100% specificity, and 100% PPV. The previous study (15) reported 90.60% sensitivity, and 82% specificity. Thus, the current study showed extremely high sensitivity and specificity.

A scoring system for the diagnosis of CLL/SLL was first developed by Matuta et al. (19). Immunophenotyping with CD43 was not used in that scoring. However, CD43 immunophenotyping is recommended by the European Consensus (20), especially for atypical and borderline cases. CD43 in FC in the current study was found to have 97.73% sensitivity, 73.91% specificity, 87.76% PPV, and 3.75 positive likelihood ratio. These values were seen to be higher than the previously reported rates of 71.80% sensitivity and 88.70% specificity in one study (21) and lower than those of another with 100% and 88.50%, respectively (22). No comprehensive study could be found in the literature of CD43 in IHC of bone marrow biopsies of CLL/SLL cases. Publications are generally in the form of case reports (23). In the current study, CD43 in IHC for CLL/SLL was found to have 93.18% sensitivity, 70.83% specificity, and 85.42% PPV, showing extremely high sensitivity.
In previous studies, the diagnostic value of sIg in CLL/

Table 3. The diagnostic performances of CD23, CD43, CD5, and LEF-1 immunohistochemical markers in CLL/SLL diagnosis

Biopsy	CD23	CD43	CD5	LEF-1	CD23+CD43+CD5+LEF-1
	% (95% Cl)	% (95% Cl)	% (95% Cl)	% (95% Cl)	% (95% Cl)
Sensitivity	90.91(78.33-97.47)	93.18(81.34-98.57)	88.64(75.44-96.21)	100.00(91.96-100.00)	95.45(84.53-99.44)
Specificity	95.83(78.88-99.89)	70.83(48.91-87.38)	70.83(48.91-87.38)	100.00(85.75-100.00)	100.00(85.75-100.00)
Positive Likelihood Ratio	21.82(3.20-148.97)	3.19(1.70-5.99)	3.04(1.61-5.72)		
Negative Likelihood Ratio	0.09(0.04-0.24)	0.10(0.03-0.30)	0.16(0.07-0.38)		0.05(0.01-0.18)
Positive Predictive Value	97.56(85.42-99.64)	85.42(75.75-91.65)	84.78(74.75-91.29)	100.00	100.00
Negative Predictive Value	85.19(69.23-93.63)	85.00(64.85-94.57)	77.27(58.89-88.97)	100.00	92.31(75.60-97.89)
Accuracy	92.65(83.67-97.57)	85.29(74.61-92.72)	82.35(71.20-90.53)	100.00(94.72-100.00)	97.06(89.78-99.64)

*CLL/SLL: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma. 95% Cl: 95% confident interval

Table 4: Performances of CD23, CD43, CD5 and CD200 in flow cytometry in the diagnosis of CLL/SLL

	CD23	CD43	CD5	CD200	CD23+CD43+CD5+CD200
	% (95% Cl)	% (95% Cl)	% (95% Cl)	% (95% Cl)	% (95% Cl)
Sensitivity	97.73(87.98-99.94)	97.73(87.98-99.94)	97.73(87.98-99.94)	97.73(87.98-99.94)	100.00(91.96-100.00)
Specificity	60.87(38.54-80.29)	73.91(51.59-89.77)	65.22(42.73-83.62)	100.00(85.18-100.00)	82.61(61.22-95.05)
Positive likelihood ratio	2.50(1.50-4.17)	3.75(1.88-7.46)	2.81(1.60-4.93)		5.75(2.36-14.01)
Negative Likelihood Ratio	0.04(0.01-0.27)	0.03(0.00-0.22)	0.03(0.00-0.25)	0.02(0.00-0.16)	
Positive Predictive Value	82.69(74.12-88.85)	87.76(78.25-93.46)	84.31(75.40-90.41)	100.00	91.67(81.86-96.40)
Negative Predictive Value	93.33(66.24-99.01)	94.44(70.69-99.17)	93.75(67.87-99.07)	95.83(76.82-99.38)	100.00(0.00-0.00)
Accuracy	85.07(74.26-92.60)	89.55(79.65-95.70)	86.57(76.03-93.67)	98.51(91.96-99.96)	94.03(85.41-98.35)

*CLL/SLL: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma. 95% Cl: 95% confident interval

SLL has been found to be limited, and it has even been thought to be more appropriate to remove it from diagnostic panels (15). In the majority of the current study cases, light chain expression was not determined. Lambda was found to be significant in respect of the median FC percentage between CLL/SLL and non-CLL/SLL cases. In cases showing Lambda clonality, the median FC percentage was statistically significantly higher at 81.36% in CLL/SLL cases than 67.50% in non-CLL/SLL cases (p=0.010).

Previous studies with FISH analysis have determined del13q most often in CLL/SLL at the rate of 33.30% (6), and similarly but at a higher rate, the most common cytogenetic finding in the current study was del13q at 48.15%.

Limitations

In this study, a method comparison was made between IHC and FC in low-grade lymphomas in adults. Appropriate panels were tried to be determined for both methods for diagnosis. However, except for CLL/SLL cases, the number of our other leukemia/lymphoma cases has been limited, as is the case all over the world.

CONCLUSION

Different panels and scoring systems have been recommended in literature to differentiate a diagnosis of CLL/SLL, which is the most frequently seen of the B cell leukemia/lymphomas causing infiltration to the bone marrow, from other B cell lymphoproliferative conditions [22, 19, 24]. One of these scoring systems is immunophenotyping with CD5, CD22, CD23, FMC7, and SmIg (24). In the current study, in the panel made with CD23(+), CD43(+), CD5(+), CD200(+) in FC, sensitivity was determined as 100% and specificity as 82.61% for CLL/SLL, and in the panel made with CD23(+), CD43(+), CD5(+), LEF-1(+) in IHC, 95.45% sensitivity and 100% specificity for CLL/SLL were determined. Such a broad comparison of IHC and FC could not be found in the literature. With these panels, the two methods had very close diagnostic values. Therefore, we recommend adding LEF-1 to the IHC panel and CD200 to the FC panel in bone marrow biopsy. Thus, both CLL/SLL can be diagnosed and differential diagnosis can be made with high accuracy from other mature B cell leukemia/lymphomas with atypical immunophenotype.

Ethics Committee Approval: The study was approved by the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (2021/429).

Informed Consent: Retrospective Study

Authorship Contributions:

Idea/Concept: ÇÖ, Design: ÇÖ, Supervision: TK, Data Collection or Processing: MŞ, FY, TK, ÇÖ, Analysis or Interpretation: YŞ, Literature Search: MŞ, Writing: ÇÖ, YŞ, MŞ, Critical Review: TK, References And Fundings: -, Materials: -.

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