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Original Research

Diagnostic Utility of Lymphoid Enhancer Binding Factor 1 Immunohistochemistry in Chronic Lymphocytic Leukaemia/ Small Lymphocytic Lymphoma

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Objective: Chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) is a common mature B-cell neoplasm composed of monomorphic small mature B cells co-expressing CD5 and CD23. Diagnosis is based on morphologic evaluation and immunophenotyping, but no specific immunohistochemical marker is availableTo evaluate the usefulness of LEF1 in the diagnosis of CLL/SLL by immunohistochemistry and in distinguishing CLL/SLL from other small B-cell lymphomas.

Methods: Previously diagnosed 120 cases of small B cell lymphomas by IHC/flowcytometry from 1st January 2017 to 31st December 2019 in the RCC, Thiruvananthapuram are included in the study. This includes thirty diagnosed cases of CLL/SLL (lymph node/ bone marrow trephine biopsies). Thirty cases of follicular lymphoma, 30 cases of mantle cell lymphoma and 30 cases of marginal zone lymphoma are also included in the study for comparison. Clinical details, morphological features and IHC already done for these cases (CD20, CD5, CD23 and cyclin D1) were analysed. IHC for LEF1 is done in all 120 cases using Rabbit (EP310) monoclonal antibody. Data is entered into excel sheet and analysis is done using SPSS software.

Result: LEF1 has 100% sensitivity, specificity, NPV, PPV, and accuracy for diagnosing CLL/SLL, with a diagnostic cut-off of 100%, compared to other immunomarkers.

Conclusion: LEF1 is a highly specific and sensitive diagnostic marker for CLL/SLL, aiding in the differential diagnosis of small B-cell lymphomas.

Introduction

Chronic lymphocytic leukaemia /small lymphocytic B cell lymphoma (CLL/SLL) is a mature B cell neoplasm and is the most common form of leukaemia of adults [1]. Diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma is primarily based on the morphological evaluation and immunophenotypic analysis, but there is no specific immunohistochemical marker available for the

diagnosis of CLL/SLL [1-3]. CLL/SLL is often a differential diagnosis when pathologists deal with small B -cell mature lymphoid infiltrates in the bone marrow, lymph nodes, and other organs [1, 4-8]. Other B-cell lymphomas comprised of primarily small lymphocytes include follicular lymphoma (FL), mantle cell lymphoma (MCL), lymphoplasmacytic lymphoma, and marginal zone lymphoma (MZL) [1, 4] [9-11]. These may be distinguished based on their typical architectural patterns and by immunohistochemistry and flow cytometry analysis [1, 12, 13]. Follicular lymphomas are positive for CD10 and Bcl6, which are markers of germinal center differentiation [1, 9, 10, 14]. Mantle cell lymphomas stain positive for Cyclin D1 and SOX11. Chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) is a B-cell lymphoma comprising of monomorphic small mature B-cells that frequently co-express CD5 and CD23 [1, 4, 15]. CLL/SLL-cells express pan-B-cell markers (CD19, CD20, CD79a, and PAX5), CD5, and CD23, and are negative for CD10, Bcl6 and cyclin D1 [4, 15, 16]. However there has been no specific immunohistochemical marker available for CLL/SLL [2, 15, 16].

Though there are well defined morphologic and immunophenotypic characteristics, diagnosis of small B-cell lymphomas is still challenging in a proportion of cases [12, 17-24]. Significant morphologic and immunophenotypic overlap has been observed between the two major types of CD5 positive B-cell lymphomas, chronic lymphocytic leukemia/small lymphocytic lymphoma and mantle cell lymphoma. Rarely SLL/CLL may be CD5 negative [20-25]. Atypical immunophenotypes may cause diagnostic challenges, particularly when the morphologic features are not classic.

Recent studies have shown that newer immunohistochemistry (IHC) marker, Lymphocyte enhancer binding factor 1 (LEF1) is positive in up to 95% of cases of CLL/SLL, and is rarely expressed in other small B-cell lymphomas [2, 3, 4, 10, 12, 16]. Lymphoid enhancer binding factor (LEF1) is a crucial transcription factor for proliferation and survival of B cells and T cells and acts via the wingless-type mouse mammary tumour virus integration site (Wnt) signalling pathway [13, 20-28] Several recent studies have demonstrated dysregulation of WNT/b-catenin signaling in chronic lymphocytic leukemia [27-41]. This pathway regulates cell fate determination during development as well as cell proliferation and survival [42-44]. The ultimate mediator of this pathway is a nuclear complex of LEF/T-cell factor (TCF) and b-catenin that work together to regulate the transcription of a variety of WNT target genes [5, 15, 16, 30, 31, 41, 45]. LEF1 is normally expressed in T and pro-B cells, and is involved in early lymphocyte development. Data on LEF1 expression by immunohistochemistry in small B-cell lymphomas are limited [2, 3, 4, 10, 12, 16]. Our study aims to assess the LEF1 expression by immunohistochemistry in chronic lymphocytic leukemia/ small lymphocytic lymphoma as well as in other subtypes of small B-cell lymphomas.

Materials and Methods

Previously diagnosed 120 cases of small B cell lymphomas by Immunohistochemistry (IHC) or flowcytometry from 1st January 2017 to 31st December 2019 in the department of Pathology Regional Cancer Centre (RCC), Thiruvananthapuram were included in the study. This includes thirty diagnosed cases of CLL/ SLL from lymph nodes, extra nodal sites and bone marrow trephine biopsies during the period 01.01.2017 to 31.12.2019. Thirty cases of follicular lymphoma, 30 cases of mantle cell lymphoma and 30 cases of marginal zone lymphoma were also included in the study for comparison.

Methodology

We retrospectively reviewed 30 cases each of CLL/ SLL, FL, MCL and MZL on biopsies from lymphnodes, extra nodal sites and bone marrow trephines which were diagnosed according to the World Health Organisation 2017classification. The corresponding blocks were retrieved from archives of Department of Pathology, RCC, Thiruvananthapuram. Cases of blocks with tissue insufficient for IHC study were excluded. Sample size was calculated using statistical formula

based on data from earlier studies and was found to be 30 cases each of CLL/SLL, FL, MCL, and MZL accounting for a total of 120 cases.

Demographic details , morphological features and IHC findings already done for these cases (CD20, CD5, BCL6, CD 10, CD23 and cyclin D1) were analysed. IHC for LEF1 was done in all these cases using Rabbit (EP310) monoclonal antibody. Data were entered into an Excel sheet, and analysis was done using SPSS software version 28.0.

IHC was done on additional tissue sections taken from the retrieved blocks using LEF1 rabbit monoclonal antibody with clone no: EP310 (ready to use). Slides were processed on the Leica Bond system (Leica Biosystems, Wetzlar, Germany). For antigen retrieval, heat-induced epitope retrieval was done using citrate buffer of pH 6 in microwave at high wattage for 10 minutes. Incubation time of the primary antibody was one hour at 37 degree Celsius in a humid chamber. Detection with 3,3’-diaminobenzidine was used.

Interpretation of LEF1 expression

Nuclear staining of at least >70% of neoplastic cells were taken as positive. Nuclear staining less than 70% were taken as negative [2,15]. The weak, moderate, and strong intensity of staining was taken as positive. All results and data were entered in Excel sheet and analysed by using appropriate statistical software SPSS version 28.0.

Statistical methods

The categorical variables will be summarized using frequencies and percentages. Continuous variables will be presented using mean and SD. The diagnostic accuracy will be assessed using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. The association between two categorical variables will be assessed using Chi-square/Fisher’s Exact test. A p-value of <0.05 is significant.

Results

The mean age of the total number of patients in the present study was found to be 58.03 years, with a standard deviation of 10.6. The highest mean age was found in the MZL group (62.8 years), and the lowest mean age was found in FL (52 years). The mean age of patients with a diagnosis of mantle cell lymphoma was 61 years. Of 30 cases of CLL/SLL, 19 (63.33%) were males and 11 (36.66%) were females. Among the 30 cases of FL, there were 19 (63.33%) males and 11 (36.66%) females.

Of the 30 cases of MCL, 26 were males (86.66%) and 4 (13.33%) were females. Among the 30 cases of MZL, there were 15 (50%) males and 15 (50%) females. There is a significant difference in the gender distribution of small B cell lymphomas. The male-to-female ratio was highest for MCL (6.5:1) and lowest for MZL (1:1).

Immunohistochemical staining for LEF1 was performed on already diagnosed 120 cases of small B-cell lymphomas which included 30 cases of chronic lymphocytic leukemia/small lymphocytic lymphomas, 30 cases of mantle cell lymphomas, 30 cases of follicular lymphomas and 30 cases of marginal zone lymphomas.

In CLL/SLL, Immunohistochemistry for LEF1 was done in 8 (26.66%) bone marrow trephine biopsies and 22 (73.33%) lymph node biopsies. In follicular lymphoma, 28 cases were from lymph node biopsies and one case each from the spleen and bone marrow. Among 30 cases of mantle cell

lymphoma, 21 (70%) were from lymph node and 6 (20%) from bone marrow trephine biopsies. Rest of the 3 cases are from the tonsil, lacrimal gland and orbit.

In marginal zone lymphoma,15 biopsies (50%) were from lymph node, 2 (6.66%) from bone marrow trephine and 4 (13.33%) from orbit.

Expression of LEF1 in CLL/SLL

All CLL cases expressed LEF1. Biopsy sites included 22 lymph nodes and 8 bone marrow trephine biopsies. The classic morphology of small lymphoid cells with scanty cytoplasm and clumped chromatin was seen in all cases.

Seventeen CLL cases showed the classic immunophenotype (CD20 +/CD5+ /CD23+ /CD10–/ cyclin D1–) (Figure 1).

Figure 1. Chronic Lymphocytic Leukaemia/Small Lymphocytic Lymphoma; (A) Lymph node showing atypical small lymphoid cells with scanty cytoplasm and clumped chromatin (200x). (B) Immunohistochemistry for CD20 positive in atypical lymphoid cells (400x).(C) Immunohistochemistry for CD5 positive in atypical lymphoid cells (400x). (D) Immunohistochemistry for CD23 positive in atypical lymphoid cells (400x).

Immunohistochemistry for CD5 was positive in all cases. Thirteen cases of CLL were negative for CD23. CD10 and cyclin D1 were negative in all cases.

Immunostaining for LEF1 demonstrated positivity in all cases of CLL (Figure 2).

Figure 2. Chronic Lymphocytic Leukaemia/Small Lymphocytic Lymphoma; (A) Immunohistochemistry for LEF1 positive in atypical lymphoid cells (400x). (B) Immunohistochemistry for Cyclin D negative for atypical lymphoid cells (200x).

Uniformly strong nuclear staining of LEF1 was seen in most of the lymphnode biopsy cases. In two lymphnode biopsies LEF1 staining was heterogeneous with subcapsular enhancement and dim positivity in poorly fixed central parts due to fixation artefact. LEF 1 showed variable and heterogenous positivity in bone marrow trephine biopsies when compared to lymphnode possibly due to acid decalcification.

Relatively stronger staining intensity was present in the prolymphocytes within the proliferation centers in comparison with the background non-transformed cells. In cases with well-definable proliferation centres, an enhancement in these centres was apparent. Six cases of CLL showed expanded proliferation centers. One case of CLL had areas of large cell transformation (Richter’s transformation) and the large cells also showed expression of LEF1.

LEF1 Expression in follicular lymphoma, mantle cell lymphoma and marginal zone lymphoma

None of the cases of follicular lymphoma, mantle cell lymphoma or marginal zone lymphomas expressed LEF1.

Among 30 mantle cell lymphomas, majority were small cell type. There was a case of pleomorphic mantle cell lymphoma and one case of blastoid variant. The diagnosis of these cases were previously confirmed by immunostaining for cyclin D1. The classic immunophenotype (CD20 +/CD5+ /CD23- / cyclin D1+) was seen in all 30 cases.

Among 30 follicular lymphomas included in this study, 4 were grade 1–2 (low grade) and 26 were grade 3A. Immunostaining for LEF1 showed that all 30 cases of follicular lymphomas were negative; Positive staining was seen only in the admixed T cells in the interfollicular areas.

A total of 30 marginal zone lymphomas were examined for LEF1 expression in this study, including 15 nodal, 2 bone marrow and 13 extra nodal marginal zone lymphoma of mucosa-associated lymphoid tissue. All cases were CD5 negative in marginal zone lymphomas. All 30 cases were negative for LEF1 in the neoplastic cells; positive staining was seen in the admixed T cells. Expression of immunomarkers in our study cases are depicted below (Table 1).

IHC Marker	CD20		CD5		CD23		BCL6		Cyclin D1		LEF1	
Expression of IHC	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
CLL/SLL	30	0	30	0	17	13	0	30	0	30	30	0
FL	30	0	0	30	4	26	30	0	0	30	0	30
MCL	30	0	30	0	0	30	0	30	30	0	0	30
MZL	30	0	0	30	0	30	0	30	0	30	0	30

Table 1. Expression of Immunomarkers in Study Case.

LEF1 as a diagnostic marker for CLL/SLL had an area under the receiver operation characteristic curve of 1.00 (100%) (Table 2 and Figure 3).

Diagnostic Test	CD20	CD5	CD23	CD10	BCL6	Cyclin D1	LEF1 (%)
Sensitivity	100%	100%	56.70%	0	0	0	100
Specificity	0	66.70%	97.80%	67.80%	66.70%	66.70%	100
PPV	25%	50%	89.50%	0	0	0	100
NPV	0	100%	87.10%	67%	66.70%	66.70%	100
Accuracy	25%	75%	87.50%	50.80%	50%	49.60%	100

Table 2. Specificity and Sensitivity of LEF1 Expression for Diagnosis of CLL in the Setting of a Small B-Cell Lymphoma.

Figure 3. Specificity and Sensitivity of LEF1 Expression for Diagnosis of CLL in the Setting of a Small B-Cell Lymphoma.

While CD20 was sensitive but not specific, CD23 was moderately sensitive with a specificity of 97.8%, and CD5 was sensitive with a moderate specificity of 66.7%. All other markers showed moderate specificity with no sensitivity. NPV, PPV, and accuracy: when compared to other immunomarkers, LEF1 showed 100% value. 17/30 (56.66%) of CLL/SLL cases were positive for CD5, CD23, and LEF1 whereas 13 (43.33%) of the cases were CD5+/CD23-/ LEF1+. The ROC curve showed that CD5 was sensitive with a moderate specificity of 66.7%, and CD23 was fairly sensitive with a specificity of 97.8%. LEF1 has therefore shown high specificity (100%) and sensitivity in comparison to other biomarkers, CD23 and CD5.

Discussion

CLL is a clonal lymphoproliferative disorder characterized by proliferation of mature B cells.

Peripheral blood examination characteristically reveals an absolute lymphocytosis usually greater than 5000/ µL with a predominance of a monotonous lymphoid cell population on the blood smear [1-3]. Lymphoid cells are characteristically small, mature-appearing lymphocytes with a narrow cytoplasmic border, a compact nucleus devoid of apparent nucleoli, and partially aggregated chromatin. Although CLL primarily involves the peripheral blood and the bone marrow, lymph node involvement is also very common. The lymph nodes are diffusely infiltrated by neoplastic lymphocytes that morphologically resemble the neoplastic cells in the peripheral blood and the bone marrow .

Diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma is primarily based on the morphological evaluation and immunophenotypic analysis [1,4-8]. Significant overlap in morphology and immunophenotype has been observed between the two major types of CD5 positive B-cell lymphomas, chronic lymphocytic leukemia/small lymphocytic lymphoma and mantle cell lymphoma[2, 4, 12, 15]. There have also been reports of CD5 expression in other B-cell lymphomas, including follicular and marginal zone lymphomas [4. 10, 14]. Additionally, chronic lymphocytic leukemia/small lymphocytic lymphoma can be CD5 negative [4, 12, 15]. These non-classical features of these atypical immunophenotypes may make a diagnosis difficult. LEF1's roles in hematopoietic development have been been limited to lymphoid lineages, where LEF-1 influences pro-B cell proliferation and apoptosis as well as T-cell development [27, 28, 30, 31]. Specific LEF-1 actions that are not dependent on Wnt signalling point to a more complex role for LEF-1 in hematopoietic tissue development [28, 30]. Among small B-cell lymphomas, lymphoid enhancer-binding factor-1 (LEF-1) has a strong correlation with CLL/SLL [4, 10, 12, 15].

Data on LEF1 expression in B-cell lymphomas are limited and are mostly from western literature and none from India [2, 3, 10, 12]. In most of the previous studies the evaluation has been through molecular or flowcytometry methods and were primarily used in research settings. Our study assessed LEF1 expression by immunohistochemistry in a series of chronic lymphocytic leukemia/small lymphocytic lymphoma and compared it with other small B cell lymphomas.

Age

In a recent study by Ahmed El-Khamessy et al. (2018) [3], the mean age of CLL and SLL cases lies between 67 and 72 years, which is slightly higher than in our study. The mean age of CLL and SLL cases in our study group is 54.86%. In our study, the mean age of the patients with MZL was 62.8 years, follicular lymphoma was 53.46 years, and MZL was 62.08%, which is comparable to the median age (59–60 years) in the western literature. There was no significant statistical difference in the mean age among our study groups of small cell lymphomas.

Gender Distribution

CLL has a male-to-female ratio of 2:1 in western literature [1-3], but it was low in our study group (1.72:1). MZL typically have a female predominance (1:1.2), which was not observed in our study (1:1).In the literature, the male to female ratio in follicular lymphoma is 1:1.7, which was slightly lower in our study (1.72:1).There was no significant statistical difference in the gender distribution among our study groups of small cell lymphomas.

LEF1 expression in study cases

Our study showed nuclear staining of LEF1 in neoplastic cells of all cases of chronic lymphocytic leukemia/small lymphocytic lymphomas, but not in other types of small B-cell lymphomas examined, including 30 cases of mantle cell lymphomas, 30 cases of follicular lymphomas and 30 cases of marginal zone lymphomas of various tissue origins. Positive staining was seen only in the admixed

T cells in all small B cell lymphomas other than CLL/SLL. Our results indicate that nuclear overexpression of LEF1 is readily detectable by immunohistochemistry in paraffin-embedded tissue and is highly associated with chronic lymphocytic leukemia/small lymphocytic lymphoma among small B-cell lymphomas. One important advantage of LEF1 is its nuclear localisation, making it less prone towards interpretational bias. Thus, LEF1 may serve as a convenient immunohistochemical marker for diagnosis and differential diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma, particularly when morphological features or immunophenotype is atypical. In all cases of CLL/SLL, FL, MCL, and MZL, the immunohistochemistry expression of LEF1 was evaluated. In 30/30 (100%) CLL study cases, positive nuclear staining was seen, which is comparable to what has been written in the literature. LEF 1 can be used in bone marrow trephine biopsy specimens, core needle biopsy specimens, lymph node excisions and other tissue biopsy specimens. Expression of LEF1 was strong and uniform in lymphnode specimens compared to heterogenous expression in bone marrow trephine biopsies in our study. Other studies didn't show any difference in staining in bone marrow and other tissues [2, 12, 15] .

In a study by Amador-Ortiz et al (2015) [12] all 25 cases of CLL showed positivity for LEF 1 and all cases of MCL, FL and MZL were negative for LEF 1. This is comparable to our study which also shows 100% sensitivity and specificity. Study by Tandon et al (2011)

[4] showed 100% positivity for LEF1 in CLL/SLL which is also comparable to our data. But specificity was low compared to our study as some of the follicular lymphomas also expressed LEF1.

Study by Menter et al. (2015) [2] showed a few LEF1-negative [39/56 (70%)] CLL cases and a few LEF1-positive cases in other small B-cell lymphomas. So sensitivity and specificity was low compared to our study. In a recently published study by Soliman et al. (2020) [16], 40/42 (95.2%) CLL/SLL cases showed positivity, with a few LEF1-positive marginal zone lymphoma cases. Also Menter et al. (2017) [15] showed a few LEF1-negative [77/80 (96%)] CLL cases and a few LEF1-positive cases in other small B-cell lymphomas. According to previous studies, the positivity for LEF1 in CLL ranges from 70% to 100% (Table 3).

Study	Cases	Total cases	LEF1 positive staining	LEF1 positivity in CLL/SLL cases 100%
Amador-Ortiz et al (2015) [12]	CLL/SLL	25	25/25	100%
	FL	6	0/6	
	MCL	4	0/4	
	MZL	4	0/4	
Menter et al (2015) [2]	CLL/SLL	56	39/56	70%
	FL	60	1/60	
	MCL	17	2/17	
	MZL	102	1/102	
Soliman et al (2020) [16]	CLL/SLL	42	40/42	95.20%
	FL	12	0/12	
	MCL	7	0/7	
	MZL	17	3/17	
Tandon et al (2011) [4]	CLL/SLL	92	92/92	100%
	FL	43	6/43	
	MCL	53	0/53	
	MZL	31	0/31	
Menter et al (2017) [15]	CLL/SLL	80	77/80	96%
	FL	30	1/30	
	MCL	10	0/10	
	MZL	3	0/3	

Table 3. Published Data on LEF1 Expression in CLL/SLL.

In conclusion, LEF1 is an accurate diagnostic marker for CLL/SLL with high specificity (100%) and sensitivity (100%) and is helpful in diagnostically challenging cases of small B-cell lymphomas. LEF1 is helpful in small B-cell lymphomas with an equivocal immunophenotype or morphology, hence it can be routinely used as an immunohistochemical marker in a diagnostic algorithm for small B-cell lymphomas.

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Scientific Approval

Study is approved by the Institutional Review Board (IRB No:12/2020/06).

Ethical Declaration

Study is approved by the Institutional Ethics Committee (HEC No:6/2021).

Authors Contribution

Vishnu V L, Simi C M, Rekha A. Nair, Jayasudha A V and Priya Mary Jacob have done study design, manuscript writing and editing. Vishnu V L, Simi C M and Preethi sara George has done statistical analysis.

Data Availability

Data is available in the institutional data base

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