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The Relevance of Additional Immunohistochemical Markers on the Differential Diagnosis of Small B-Cell Lymphomas: A Case-control Study
Küçük B Hücreli Lenfomalarda Ek Immünhistokimasal Belirteçlerin Ayırıcı Tanıdaki Yeri: Bir Vaka-kontrol Çalışması

Hale Kıvrak[1], Seher Yüksel[1], Can Ateş[2,5*], Mustafa Merter[3,4*], Gülşah Kaygusuz[1], Muhit Özcan[4], Işınsu Kuzu[1]

[1]Ankara University Faculty of Medicine, Department of Pathology, Ankara, Turkey
[2]Aksaray University Faculty of Medicine, Department of Biostatistics and Medical Informatics, Aksaray, Turkey
[3]Fırat University Faculty of Medicine, Department of Hematology, Elazığ, Turkey
[4]Ankara University Faculty of Medicine, Department of Hematology, Ankara, Turkey
[5]Ankara University Faculty of Medicine, Department of Biostatistics, Ankara, Turkey

Hale Kıvrak, M.D., Ankara University Faculty of Medicine, Department of Pathology, Ankara, Turkey
karadag.hale@gmail.com
+90 545 494 94 14

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Abstract

Objective: Clinical and pathological differential diagnosis of small B-cell lymphomas (SBCLs) is still controversial and may create difficulty with their overlapping morphology, phenotype, differentiation to plasma cells. We aimed to examine the expression of the markers ımmune receptor translocation -associated protein 1 (IRTA1), myeloid cell nuclear differentiation antigen (MNDA), lymphoid enhancer-binding factor-1 (LEF1) and stathmin1 in SBCL cases involving different sites which may have plasma cell differentiation.

Materials and Methods: We studied 154 involved tissue samples, from 116 patients and evaluated the staining distribution of the markers. The expressions were evaluated on 21 chronic lymphocytic leukemia/ lymphoma (CLL/SLL), 7 follicular lymphoma (FL), 14 nodal marginal zone lymphoma (NMZL), 17 extranodal marginal zone lymphoma (ENMZL), 55 splenic marginal zone lymphoma (SMZL), 22 marginal zone lymphoma -not otherwise specified (MZL-NOS) and 18 lymphoplasmacytic lymphoma/Waldenström's macroglobulinemia (LPL/WS) cases by immunohistochemistry.

Results: The results confirmed that LEF1 was the most sensitive and specific markers for CLL/SLL and Stathmin for FL ($p < 0.001$). MNDA and IRTA1 were useful markers to distinguish marginal zone lymphomas.

Conclusion: Our results suggest LEF 1 for CLL/SLL and Stathmin for FL are reliable markers. LEF1, MNDA, Stathmin1 and IRTA1 are helpful with other routinely used immunohistochemical markers in a diagnostic algorithm considering their limitations.

Keywords: Small B cell lymphoma, MNDA, LEF1, Stathmin1, IRTA1

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Özet

Amaç: Küçük B hücreli lenfomaların (SBCL'lar) klinik ve patolojik ayırıcı tanısı halen tartışmalıdır ve örtüşen morfolojileri, fenotipleri ve plazma hücresine farklılaşmaları nedeni ile zorluk yaratabilir. Bu çalışmada plazma hücre farklılaşması gösteren farklı lokalizasyonlardaki SBCL'larda, immün reseptör translokasyonu ilişkili protein 1 (IRTA1), myeloid hücre farklılaşma antijeni (MNDA), lenfoid güçlendirici bağlama faktörü-1 (LEF1) ve stathmin1 belirteçlerinin ekspresyonu araştırılmıştır.

Gereç ve Yöntem: Çalışmada 116 hastadan alınmış olan 154 farklı dokuda belirteçlerin boyanma dağılımları incelenmiştir. Belirteçlerin ekspresyonları 21 kronik lenfositik lösemi /küçük lenfositik lenfoma (KLL/SLL), 7 foliküler lenfoma (FL), 14 nodal marjinal zon lenfoma (NMZL), 17 ekstranodal marjinal zon lenfoma (ENMZL), 55 splenik marjinal zon lenfoma (SMZL), 22 marjinal zon lenfoma-başka türlü sınıflandırılamamış (MZL-NOS) ve 18 lenfoplazmasitik lenfoma (LPL/WM) örneğinde immünhistokimyasal olarak değerlendirilmiştir.
Bulgular: Sonuçlar, LEF1'in KLL/SLL için ve Stathmin1'in FL için hassas ve spesifik belirteçler olduğunu (p değeri<0.001); MNDA ve IRTA-1'in marjinal zon lenfomalarını ayırt etmek için yararlı belirteçler olduğunu gösterdi.
Sonuç: Sonuçlarımız LEF1'in KLL/SLL için ve Stathmin-1'in FL için güvenilir belirleyiciler olduğunu göstermektedir. LEF1, MNDA, Stathmin1 ve IRTA1, tanısal bir algoritma içinde kısıtlılıkları dikkate alınarak kullanıldığında, rutin olarak kullanılan diğer immünhistokimyasal belirteçlere yardımcıdır.

Introduction

Small B-cell lymphomas (SBCLs) entities present indolent clinical bahaviour which may not be treated at the initial stages. But they are therotically incurable diseases and may progress or transform to agressive lymphoma during their course. Chronic lymphocytic leukemia/ lymphoma (CLL/SLL), follicular lymphoma (FL), nodal marginal zone lymphoma (NMZL), extranodal marginal zone lymphoma (ENMZL), splenic marginal zone lymphoma (SMZL), lymphoplasmacytic lymphoma/Waldenström's macroglobulinemia (LPL/WM), mantle cell lymphoma (MCL) are the lymphoma entities in this category [1]. Mantle cell lymphoma should be excluded from the group of SBCLs with its naïve B cell origin, clinical behaviour and different treatment approaches. Differential diagnosis between SBCLs could be challanging. Clinical and radiological findings with histomorphological, immunohistochemical and molecular data should be evaluated for correct diagnosis.
The molecular characteristics support the differential diagnosis such as IGH/BCL2 rearrangement in FL, MYD88 mutation in LPL/WM or IGH/BCL1 rearrangement causing cyclin D1 expression in MCL[1]. On the other hand, some markers such as aberrent CD5 expression which is often expected for CLL/SLL and MCL may also be found at very low rates in MZLs and LPL/WM based on flow cytometry [2]. Diagnosis of MZL could be challanging since there is no specific marker for the neoplastic cells and the diagnosis needs ruling out the other entities. The clinical presentations such as splenomegaly in SMZL and some phenotypic and molecular hallmarks for some cases as BIRC3/MALT1, IGH/MALT1, IGH/BCL10 translocation in ENMZL and NOTCH2, KLF2 mutations in SMZL may be helpful on differential diagnosis [3,4]. But these molecular findings are not as characteristic and frequent as BCL2 translocation for FL or BCL1 translocation for MCL. On daily clinical practice, a diagnostic approach by morphology and immunophenotyping is crucial. However, for the cases showing atypical presentation, unexpected phenotypic characteristics, overlapping morphologic features and especially restrictions related with the amount of tissue may cause misdiagnosis. All SBCLs may be accompanied by plasma cells in different proportions. Plasma cell differentiation with or without paraproteinemia could create difficulty in differential diagnosis of SBCLs. Especially if the first examined diagnostic material is bone marrow or a core biopsy, the diagnostic approach may be more challenging. Flow cytometry is very helpful but may not be available for every case. These circumstances reveal that in addition to the standard antigens, there is a requirement for alternative

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supportive markers solving the diagnostic problems of difficult SBCL cases. Individual studies reveal the role of several antigens expressed on different maturational and functional steps of B cells as well as the lymphomas originating from these cells.

Myeloid cell nuclear differentiation antigen **(MNDA)** is a human granulocyte-monocyte/macrophage specific nuclear protein detected initially in the late myeloblast and monoblast stages of differentiation[5]. In addition to acute and chronic myeloid leukemia; its expression was also detected in MCL, diffuse large B cell lymphoma, hairy cell leukemia and CLL/SLL[6,7]. MNDA is accepted as putative marginal zone marker with immune receptor translocation -associated protein 1 (IRTA1)[8]. **CD27**; 55 kDa homodimer CD27 is a member of the tumor necrosis factor receptor family, a key marker for memory B cells and induce their differentiation to plasma cells[9]. It is expressed at different rates in neoplastic cells of all types of MZLs and other SBCLs [10,11]. Lymphoid enhancer-binding factor-1 (**LEF1**) is a nuclear transcription factor and downstream mediator of the Wnt/β-catenin signaling pathway which provide proliferative signals for most of the progenitor cells in both the B and T cell lineage[12,13]**.** LEF1 positivity in the SBCLs is thought to support the diagnosis of CLL/SLL [14,15,16]. **IRTA1** gene is located on chromosome 1q21, thought to be involved in B cell–mediated immune responses and cell migration. It is selectively expressed in marginal zone B cells therefore is considered as a marginal zone marker like MNDA but has lower sensitivity and higher specificity compared to MNDA [8]. In some studies, IRTA1 is used as a marker for distinguish MALT lymphoma and NMZL from other SBCLs[17,18]. **Stathmin1(STMN1)** is a cytoplasmic protein also known as Oncoprotein 18. Its expression was detected in FL especially when germinal centre markers such as CD10 and/or Bcl-6 were negative[19]. It was reported as very helpful in the distinction between FL and MZLs in skin [20].

In this study we aimed to screen the distribution of MNDA, IRTA1, LEF1, CD27, STMN1 expression on various tissues involved by SBCLs to screen their value on differential diagnosis.

Materials and Methods

Case selection and clinical data: The cases studied were retrieved from the files of our department, diagnosed as one of the SBCLs, between the years 2006-2015.

Total of 517 tissue samples involved by SBCLs has been re-evaluated by their prior H&E and immunohistochemical stained slides by two authors (HK, IK). The diagnosis was made according to the criteria described in 2017 WHO classification without considering the previous diagnosis. Among these 517 cases, 154 tissue samples from 116 patients were selected based on the availability of adequate tissue on paraffin blocks with their other laboratory findings and clinical follow-up. The amount of plasma cells among the neoplastic lymphocytes were also considered. Plasma cells were inherently accompanied by many MZLs. There were also varying proportions of accompanying plasma cells in 2 of CLL/SLL and 5 of FL cases.

Patients in the MZL group were re-evaluated with their clinical findings, such as; organomegaly, B symptoms as well as the available laboratory findings such as; flow cytometry, mutation status, hemogram and protein electrophoresis. They are re-grouped as SMZL, NMZL, EMZL or MZL-NOS if the specification was not possible. Since our institude is a reference center, we were able to examine a considerable amound of splenectomies involved by SMZLs (n:32) and also synchrone involved bone marrow (n:8) and abdominal lymph nodes (n:5) of some of these cases (n:16). which gave the opportunity to compare the expression on different involved sites.

Immunohistochemistry: LEF1, MNDA, CD27, IRTA1 and STMN expressions were analyzed on formalin-fixed paraffin-embedded tissue sections by using the antibodies listed on Table 1. Ventana Roche Automated system (Ventana Medical Systems-Roche ABD) and

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secondary visualization kits listed were performed according to the manufacturer's instructions. Appropriate positive and negative controls were checked for each antibody.
Interpretation of the stainings: MNDA expression was evaluated as positive or negative according to the amount of positive neoplastic B cells. The strongly stained monocyte and macrophages in the microenvironment are used as internal positive control. The treshold more than 15% was considered as positive staininig for neoplastic B cell. The staining for **IRTA1** is considered as positive when neoplastic cells are making small groups with more than 3 cells or sheets as described in previous studies [21]. **LEF1, STMN1 and CD27** were considered as positive when more than 20% of the neoplastic cells were expressing these markers.
Distribution of the expression patterns of the antigens on normal tissues and cells and the internal positive controls are summarized in Table 2.

Statistical Analysis

Statistical Package for Social Sciences program version 20 for Windows was used. Continuous variables were given as median (min-max) and categorical variables were given as percentage. Mann Whitney U test was used to compare continuous variables between two independent groups. Chi-Square and/or Fisher's Exact tests were used in the analysis of categorical variables. For all statistical comparisons where type-1 error level (α) is below 5% ($p < 0.05$) was accepted as statistically significant.

Results

In all the diagnostic groups the mean age was 63.75 years (range 34–90). The mean ages of the patients were consistent with the expected age distribution compared to WHO 2017 criteria. There were no gender differences for any diagnostic groups ($p > 0.05$).
Twenty-two MZL samples were reclassified as MZL-NOS due to the involved tissue, morphology, phenotype laboratory and clinical features which did not help for description of nodal, splenic or extra nodal types. Nine of 18 cases of LPL/WM were confirmed by MYD88 mutation results. The distribution of the cases according to the diagnostic groups and localization properties are summarized in Table 3.

Immunohistochemical Analysis

Results of immunohistochemical (IHC) analysis are detailed of Table 4.

LEF1 expression

Immunohistochemical staining for LEF1 was performed on 154 samples, including 21 CLL/SLL, 7 FL, 18 LPL/WM, 55 SMLZ, 14 NMZL, 17 ENMZL and 22 MZL, NOS. Twenty-One samples involved by CLL/SLL (13 bone marrow and 8 lymph node), LEF1 immunostaining demonstrated uniformly strong nuclear positivity on the neoplastic cells in 10 of 21 cases (47.6%) (p: <0.001) (Figure 1, Table 4). Only 1 of 10 LEF1 positive patients (10%) presented with atypical phenotype (CD5 and/or CD23 negative). Two of the LEF1 negative cases were presenting monotypic plasma cell differentiation. There were no significant differences between LEF1 staining patterns for CLL/SLL cases involving different sites on bone marrow or lymph nodes. LEF1 is expressed in 9% (2/22) of the MZL-NOS cases. We could not be able to compare the T cells by CD3 staining in one case due to the loss of the foci on serial sections. LEF1 was negative in all other lymphomas.

MNDA and CD27 Expressions

MNDA and CD27 expressions were parallel especially for MZL cases. The expression of these two markers in all diagnostic groups were significant in MZLs (P= 0.02) (Figure 2) and CLL/SLL (p=0.019) (Figure 1) (Table 4).
MNDA expression on background macrophages and monocytes was used as internal positive control. Especially in the bone marrow samples the less intense staining on neoplastic B lymphocytes was considered for the correct interpretation and helpful for avoiding form overinterpretation (Figure 1).

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In 69% of SMZL, 78.6% of the NMZL, 64.7% of ENMZL and 63.6% of the MZL-NOS cases, MNDA expression was significant. Whereas 16 of 21 (76%) CLL/SLL, 5 of 18 (27.7%) LPL/WM and 1 of 7 (14%) FL cases MNDA was also expressed. Four of 5 MNDA positive LPL cases confirmed by their MYD88 mutation status.
Considering the bone marrow infiltrations of different diagnostic groups MNDA expression was observed in 18 of 24 SMZL (75%), 8 of 12 MZL-NOS (66.6%), 2 of 3 NMZL (66.6%) cases. The percentage and intensity of MNDA expression in MZL cases were similar in all involved tissues.

STMN1 Expression

STMN1 was expressed in 5 of 7 (71.4%) FL, 1 of 55 (1.8%) SMZL, 1 of 18 (5.6%) LPL/WM, 2 of 22 (9.1%) MZL-NOS, 1 of 7 (7.1%) NMZL and 2 of 17 (11.7%) ENMZL cases (Table 4). The number of FL cases in this study was limited due to the selection of the plasma cell rich cases. STMN1 expression was only significant in FL cases ($p<0.001$) compared with other entities (Table 4). Interestingly we observed striking STMN1 expression in 57.1% of CLL/SLL cases. The staining was selectively restricted on the para-immunoblastic cells but not on the small cell component (Figure 2).
STMN1 was expressed in two of the CD10 negative FL cases, which of one was also BCL2 negative. The FL cases were accompanying various amounts of plasma cells in this series and STMN1 was negative on these accompanying plasma cells. STMN1 expression was strictly related with the high proliferating and transformed cells in other lymphoma cases (Figure 2). The 2 STMN1-negative FLs were both CD10 and Bcl2 positive. They were histological grade 1 or 2 and had a low Ki67 proliferation index (<10%).

IRTA1 Expression

The localizations of 17 ENMZL samples are summarized Table 3A. IRTA1 was expressed in 1.8% (1/55) of SMZL, 4.5% (1/ 22) of the ML-NOS, 41.2% (7/17) ENMZL and 35.7% (5/14) of NMZL cases (Figure 3) (Table 4). IRTA1 expression was statistically addressing NMZL and ENMZL (p: <0.001). We did not observe IRTA1 expression in concomitant plasma cells in any diagnostic group.
IRTA1 expression was observed only in one of 55 SMZL cases. This case showed large cell transformation in the splenectomy material and involving the bone marrow with its small cell component. IRTA1 expression was present on bone marrow neoplastic B cell population but not in the spleen.

Plasma Cell Differentiation

Plasma cells were expressing CD27 in all diagnostic groups and were negative with the other markers (Figure 3).

Discussion

The differential diagnosis of small B-cell lymphomas could be challenging especially if they present atypical phenotype, overlapping features and plasmocytic differentiation in addition to the limitations of the biopsy samples [1,22].
MZLs less frequently express CD5 and do not express CD10. These are helpful for differential diagnosis from FL and CLL/SLL, which also have more typical immunophenotypic and molecular features. However, distinguishing follicular colonization in MZL from marginal zone differentiation in FL is a particularly challenging diagnostic dilemma [23]. Only ENMZL is a well-defined entity in terms of clinicopathologic presentation, genetics and treatment. NMZL ve SMZL share more similar morphology and molecular alternations [4].
LPL/WM is a relatively rare, characterized by immunoglobulin-M producing B cells and plasma cells, usually involving BM and sometimes lymph nodes or spleen [1]. However, neither morphology nor IgM paraproteinemia can provide a clear distinction from other SBCLs [24,25]. About %20 of SMZL may produce IgM heavy chain and plasma cell

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differentiation [26]. Although it is not specific, MYD88 L265P mutation is highly suggestive for LPL and should be performed for diagnosis as a standard. But this mutation could also be seen in a small proportion of other SBCLs [22]. In our study MYD88 mutation data is obtained from hospital records of half of the patients examined. Plasmacytoid/plasmocytic differentiation and monoclonal component are frequent in SMZL and NMZL. Therefore, these two entities can be misdiagnosed as LPL. In this study no statistically significant expression was detected with any of studied markers in the LPL/WM patients. 4 of 5 MNDA positive LPL were also confirmed by positive MYD88 mutation results.

LEF1 expression was detected in 70-100% in CLL/SLL and 18-38% in transformed diffuse large B cell lymphoma cases in different series. Minor positivity was detected in FL, MCL, LPL and low grade and high grade MZLs. The cut-off value of LEF1 expression was given as 10% for diagnostic support of CLL with the sensitivity of 92% and specificity of 70% in other studies [15,16].

Similar to the previous studies, we demonstrated the role of LEF1 as a specific and reproducible diagnostic marker for CLL (p: <0.001). The loss in LEF 1 expression was mentioned in relation with plasma cell differentiation in CLL/SLL [27]. We also seen LEF1 negative cases in our series, whereas only two of them were accompanied by monotypic plasma cells.

In lymphoid tissue most of the MNDA positive cells were located in the marginal zone and a few were in mantle zone[7]. Considering the results of the three previous studies, MNDA is expressed in many SBCLs but with the highest rates in MZLs [7,23,28].

Another point should be considered for MNDA is, its weak nuclear expression on the naive B cells of the primary follicles. This situation should be kept in mind especially on evaluating small biopsies [29]. Another important finding of our study is the significant expression of MNDA in CLL samples (p: 0.019). There are also similar results reported one of which mentioning the expression is related with better prognosis [30].

CD27 is expressed on activated and memory B cells of marginal zone, B1 cells, memory T cells and highly on plasma cells. In parallel with MNDA, CD27 expression was statistically significant in MZLs. This situation is consistent with two reasons. First, the marginal zone region may contain plasma cells which express CD27 [31]. Secondly, CD27 positive postgerminal centre memory cells are located in this area [32]. It's known that majority of B lymphocytes in marginal zone localization are IgM (+), IgD (-) and CD27 (+). The high expression of CD27 and MNDA in MZLs can be considered as a hallmark of a memory B cell. While MNDA expression was not observed in plasma cells accompanying neoplastic lymphocytes, CD27 has a role in plasma cell differentiation and can be widely expressed.

Selective IRTA1 expressing B cells in normal toncil are located in intraepithelial and subepithelial areas as well as within the dome epithelial cells of Peyer patches. IRTA1 expressing memory B cells also express CD27 in lymph node[33]. In our study, IRTA1 expression was found statistically significant in NMZL ve ENMZL cases same as the literature. There is one study indicates that IRTA1 antibody is useful to distinguish MALT lymphoma from other low-grade B-cell lymphomas[17]. Although it is a useful marker for addressing MZL, according to our results its sensitivity is low. There is one study indicating that IRTA1 antibody is useful to distinguish MALT lymphoma from other low grade B cell lymphomas[17]. Although different positive cut-off values are given in the literature, it is expressed at a higher rate in MZLs especially in ENMZL and NMZL cases. Similarly, IRTA1 expression was not seen in concominant plasma cells[18]. The results indicate that it can be used as a marginal zone marker, especially distinction FL from MZLs in small biopsies or in cases which cellular detail cannot be assessed due to artifacts [8].

The diagnosis of FL is based on immunohistological evidence of neoplastic GC cells expressing bcl-2, bcl-6, and CD10, and molecular detection of the frequently seen t(14;18)

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abnormality. Immunohistochemistry and morphology are helpful for the differential diagnosis. The differences of the sensitivity Bcl2 antibody clones may also affect the diagnostic challenges sometimes. A reliable germinal center B cell marker would be helpful in the circumstances if FISH could not be available. In our study, we confirmed the significance of STMN1 expression in FL cases compared to other SBCLs. Similar to the findings in the study by Marafiotti et all, STMN1 seems to be a candidate marker solving the differential diagnostic problems for FL [19]. Similarly, as given in the suplementary list of Marafiotti series, we observed STMN1 expression in 12 of 21 CLL cases, but selectively on para immunoblastic cells. Similar to the CLL, STMN1 expression on the other lymphoma cases in our study were strictly related to the transformed cells. The two of our FL cases without STMN1 expression were low grade (grade 1-2) with very low proliferation.

Study Limitations

MYD88 mutation only confirmed for half of the LPL/WM cases due to the limitations of the archived bone marrow biopsy specimens. The diagnosis of LPL/WM was confirmed with either by flow cytometry or immunohistochemistry and clinical characteristics for the cases which molecular confirmation could not be possible.

Conclusion

New markers are helpful for differential diagnosis of SBCL cases in which if specific molecular changes could not be identified or limitations due to the size of the biopsies is present. LEF1 and STMN1 are sensitive and specific markers for CLL/SLL and FL respectively. MNDA and IRTA1 are useful markers for suggesting marginal zone phenotype. The sensitivity of IRTA 1 is lower compared to MNDA, but when it is expressed, this finding could be helpful to exclude SMZL from other MZL entities. Our results suggest LEF 1 for CLL/SLL and Stathmin for FL are reliable markers. LEF1, MNDA, Stathmin1 and IRTA1 are helpful with other routinely used immunohistochemical markers in a diagnostic algorithm (Algorithm) considering their limitations.

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Ethics

All patients provided written informed consent. All authors ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Ethics Committee of Ankara University School of Medicine awarded approval for this study (approval number: I3-596-14).

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Contributor's Statement

• HK and IK design of the study and acquisition of data
• HK, SY, IK, MM and MO collected the laboratory and clinical data for the research
• GK contributed STMN1 supply
• CA statistical data analize
• All authors drafted the manuscript, figures and tables.

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Figure 1: A CLL/SLL case characterised by monomorphic small mature B cells (A, B) is showing typical coexpression of CD5 (C) and CD23 (D) with nuclear LEF1 (E) positivity by IHC. A SMZL case with plasma cell differentiation (F). The neoplastic B lymphoid cells were expressing nuclear MNDA (G, H) and cytoplasmic CD27 (I, J). The staining intensity of macrophages as internal positive control is strikingly strong (arrow) comparing to the neoplastic B cells (star) (K). An case of CLL/SLL (L, M) expressing nuclear MNDA (N, O) and cytoplasmic CD27 (P, Q).

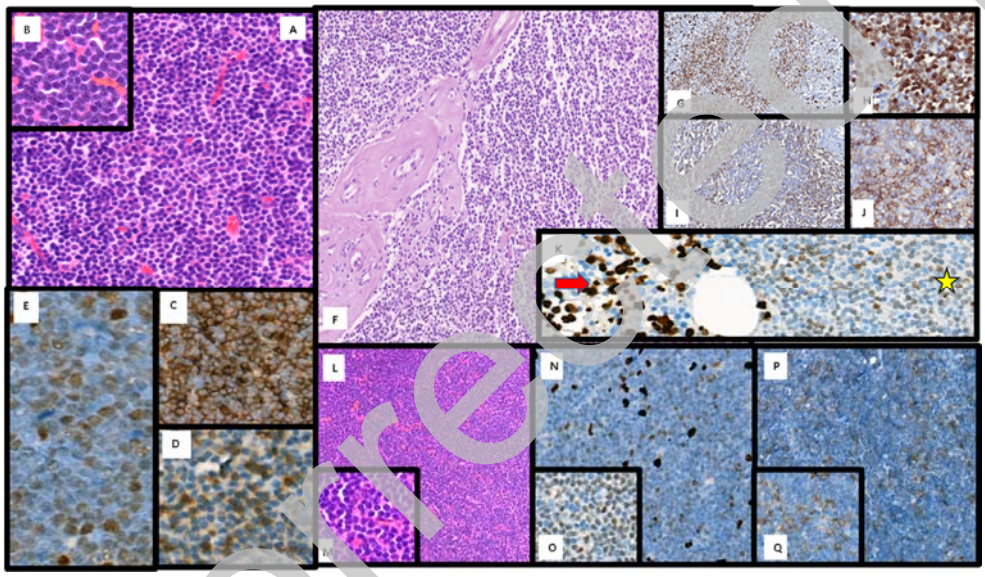


Figure 2: A case of FL with closely packed neoplastic follicles showing back-to-back pattern (A) with strong cytoplasmic STMN1 expression (B, C) by IHC. A CLL/SLL case (D, E) is demonstrating selective STMN1 expression on paraimmunoblasts whereas small cells are negative (F, G). A case of SMZL (H) with transformation is showing small (star) and a large cell (+) area, confirmed by the striking difference of Ki67 (I) proliferation status. STMN expression is prominent in the large cells (+) (J), compared to the small (star) (J) cells.

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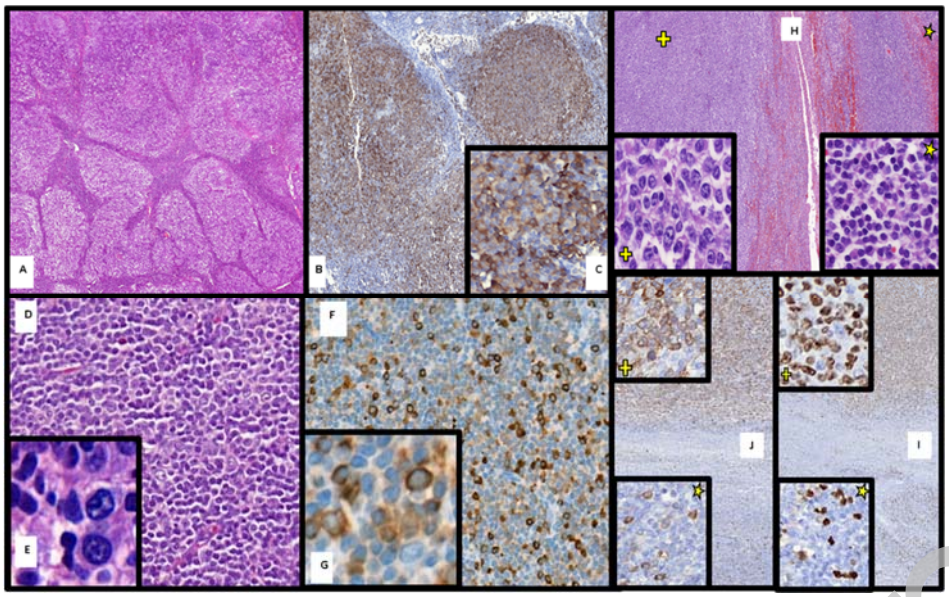


Figure 3: A Case of ENMZL of lung with neoplastic lymphoid cells infiltrating interstitial areas (A). Diffuse cytoplasmic IRTA1 (B, C) and pale nuclear MNDA expressing neoplastic lymphoid cells with scattered strong positive histiocytes (arrow) at the background (D-E). A case of NMZL (F, G) with striking sinusoidal infiltration pattern showing strong cytoplasmic IRTA1 (H, I) expression. A case B cell lymphoma with predominant monotypic plasma cell differentiation (J) with striking infiltration of interfollicular areas (K). Only CD27 (L, M) expression was observed in the neoplastic plasma cells. STMN1 (N, O) was positive on the reactive residual germinal centers.

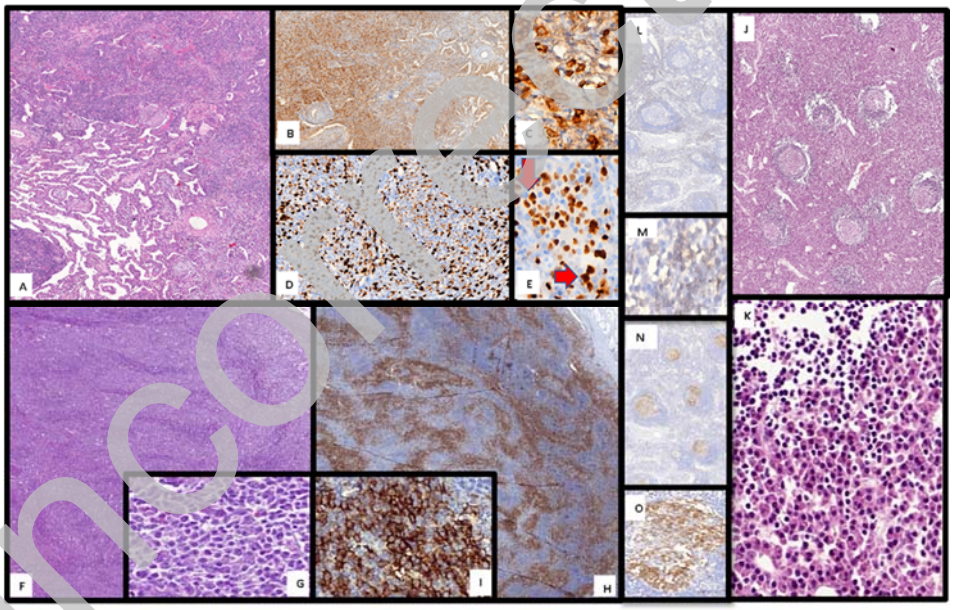


Figure 4 (Algorithm): The algorithm of expression of the markers in SBCLs in our series is summarized regarding the differential diagnosis.

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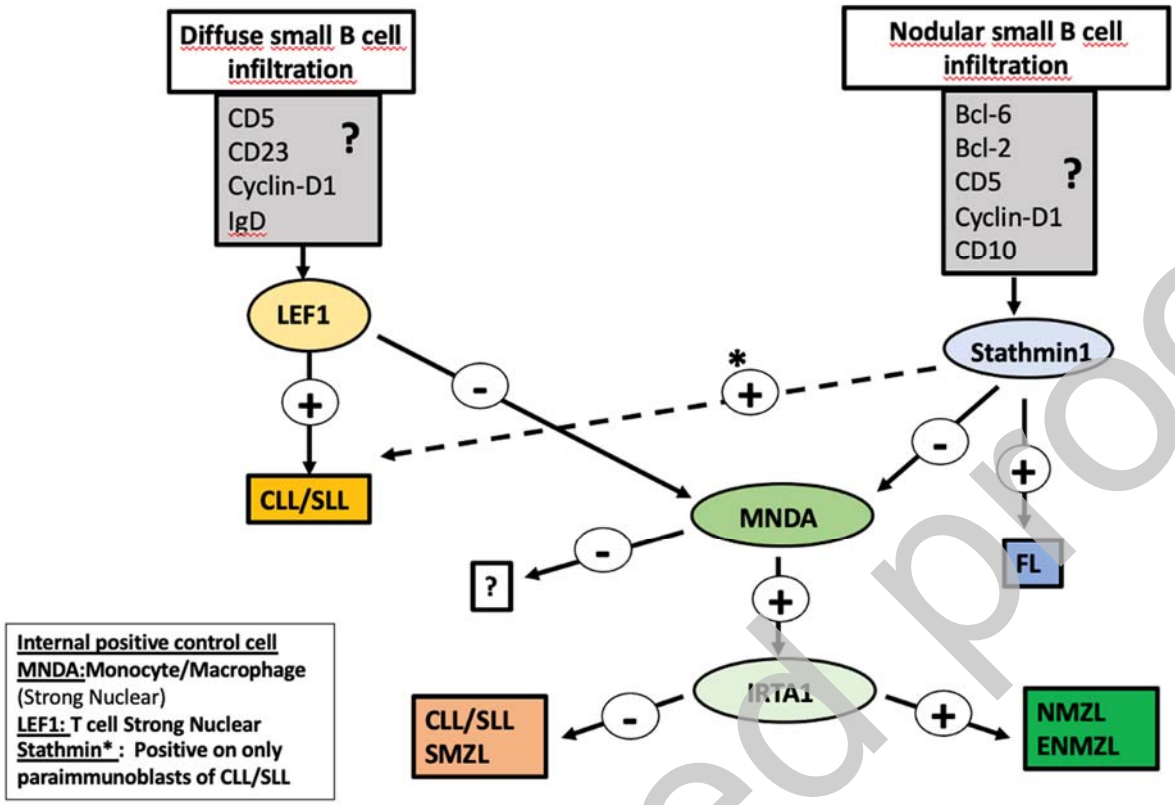


Table 1. Technical details of the antibodies and the immunohistochemistry procedures

Antibody (Ab) against	Antigen retrieval / duration (min)	Ab incubation (min)	Clone	Dilution	KIT	Supplier
LEF1	EDTA/ 30	40	Rabbit Monoclonal EPR2029Y	1/200	Biotin free OptiView DAB IHC Detection Kit	Abcam
MNDA	EDTA/ 60	60	Mouse Monoclonal 253A	1/5	Biotin free OptiView DAB IHC Detection Kit	Prof. Miguel Piris
CD27	EDTA/ 60	60	Mouse Monoclonal 137B4	1/30	Biotin free OptiView DAB	Acris

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					IHC Detection Kit	
IRTA1	EDTA/ 60	40	Murine Monoclonal IRTA1	1/10	Biotin free OptiView DAB IHC Detection Kit	Prof. Brunangelo Falini
STMN1	EDTA/ 60	32	Polyclonal	1/250	İndirect Straptavidin biotin iVIEW DAB Detection Kit	Abcam
LEF1 (lymphoid enhancer-binding factor-1), MNDA (myeloid cell nuclear differentiation antigen), IRTA1 (immune receptor translocation -associated protein 1), STMN1 (stathmin 1), Min: Minute						

Table 2. Expression characteristics of the target antigens in normal hematolymphoid tissues.

ANTIGEN	**LEF1**	**MNDA**	**CD27**	**STMN1**	**IRTA1**
LOCALIZATION	Nucleus	Nucleus	Cytoplasm	Cytoplasm	Cytoplasm
TONSIL	T cell	MZ cells Monocyte Macrophages	GC and MZ B cells	GC cells	Underneath and within the tonsil epithelium
SPLEEN	T cell	MZ cells Monocyte Macrophages	GC and MZ B cells	GC cells	-
BONE MARROW	T cell	Myeloid cells Monocyte Macrophages	Plasma cells	Myeloid and erythroid precursors	-
LYMPH NODE	T cell	MZ cells Hystiocyte Macrophages	GC and MZ B cells	GC cells	Monocytoid B cells, Endothelial cells, sinusoidal cells
LEF1 (lymphoid enhancer-binding factor-1), MNDA (myeloid cell nuclear differentiation antigen), IRTA1 (immune receptor translocation -associated protein 1), STMN1 (stathmin 1), Germinal center (GC), Marginal zone (MZ)					

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Table 3. The distribution of cases according to the diagnostic groups and the involved sites.

DIAGNOSIS	n	%	SPLEEN n (%)	BONE MARROW n (%)	LYMPH NODE n (%)	EXTRANODAL SITES* n (%)
SMZL	55	36,1	23 (41,8)	24 (43,6)	8 (14.5)	0
LPL/WM	18	11,6	0	17 (24,3)	1 (5,6)	0
MZL-NOS	22	14,2	0	12 (54,5)	9 (40,9)	1 (4,5)
NMZL	14	9	0	3 (22,4)	10 (71,4)	1 (7,1)
ENMZL	17	11	0	0	1 (5,9)	16 (94.1)
FL	7	4,5	0	1 (14,3)	6 (85,7)	0
CLL/SLL	21	13,5	0	13 (61,9)	8 (38,1)	0
TOTAL SAMPLES	**154**	100	23	70	43	18

SMZL (splenic marginal zone lymphoma), LPL/WM (lymphoplasmacytic lymphoma/Waldenström's macroglobulinemia), MZL-NOS (marginal zone lymphoma -not otherwise specified), NMZL (nodal marginal zone lymphoma), ENMZL (extranodal marginal zone lymphoma), FL (follicular lymphoma), CLL/SLL (chronic lymphocytic leukemia/ lymphoma)

*: Biopsies taken from the extranodal sites are:
- MZL-NOS case: peripancreatic soft tissue
- NMZL case: upper arm soft tissue
- ENMZL cases: 4 skin biopsies, 1 stomach, 3 lung, 2 tonsil, 2 salivary gland, 3 periorbital soft tissue, 1 larynx biopsy

Table 4. The immune profile of the diagnostic groups by the target antigens.

Diagnosis	n	LEF1 % (n)	MNDA % (n)	CD27 % (n)	STMN1 % (n)	IRTA1 % (n)
SMZL	55	0 (0)	69 (38)	50.9 (28)	1.8 (1)	1.8 (1)
LPL/WM	18	0 (0)	27.7 (5)	27.7 (5)	5.6 (1)	0 (0%)
MZL-NOS	22	9.1 (2)	63.6 (14)	81.8 (18)	9.1 (2)	4.5 (1)
NMZL	14	0 (0)	78 (11)	71.4 (10)	7.1 (1)	35.7 (5)
ENMZL	17	0 (0)	64.7 (11)	70.6 (12)	11.7 (2)	41.2 (7)
FL	7	0 (0)	14 (1)	42.9 (3)	71.4 (5)	0 (0%)
CLL/SLL	21	46.7 (10)	76 (16)	62 (13)	57.1 (12)**	0 (0%)
P VALUE *		<0.001 (for CLL)	0.02 (for MZL and CLL)	0.019 (for MZL and CLL)	<0.001 (for FL)	<0.001 (for NMZL and EMZL)

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SMZL (splenic marginal zone lymphoma), LPL/WM (lymphoplasmacytic lymphoma/Waldenström's macroglobulinemia), MZL-NOS (marginal zone lymphoma -not otherwise specified), NMZL (nodal marginal zone lymphoma), ENMZL (extranodal marginal zone lymphoma), FL (follicular lymphoma), CLL/SLL (chronic lymphocytic leukemia/ lymphoma), LEF1 (lymphoid enhancer-binding factor-1), MNDA (myeloid cell nuclear differentiation antigen), IRTA1 (immune receptor translocation -associated protein 1), STMN1 (stathmin 1)
* p values for markers are mentioned only for significant entities. (**) STMN1 positivity was detected only on scattered para immunoblastic cells of CLL/SLL cases.

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