Signet-ring cell lymphoma: clinicopathologic, immunohistochemical, and fluorescence in situ hybridization studies of 7 cases

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Keywords: Signet-ring cell lymphoma; Immunohistochemical; Fluorescence in situ hybridization

1 Introduction

Signet-ring cell lymphoma (SRCL) is a rare morphologic variant of non–Hodgkin lymphoma (NHL). The lymphoma cells typically contain abundant cytoplasmic inclusions and the nuclei may be displaced to the periphery of the cells. Although it was initially reported as a rare morphologic variant of follicular lymphoma (FL), SRCL has to date been described in most types of NHL, mostly as single-case reports and including both cutaneous and systemic NHL, such as FL, small lymphocytic lymphoma, lymphoplasmacytic lymphoma, marginal zone lymphoma of mucosa-associated lymphoid tissue, diffuse large B-cell lymphoma (DLBCL), T-cell lymphoma and anaplastic large cell lymphoma, and plasma cell myeloma [1]. Signet-ring cell lymphoma commonly involves lymph nodes, but has also been reported in extranodal tissues including the skin, gastrointestinal tract, salivary gland, breast, central nervous system, and bone marrow [1-16]. It may pose a diagnostic challenge or even be misdiagnosed, especially in the absence of an extensive immunohistochemical (IHC) study and flow cytometric analysis due to a limited needle biopsy sample [6,17].

We report 7 cases of SRCL from 6 patients with detailed clinicopathologic studies including extensive IHC study and fluorescence in situ hybridization (FISH) analyses for gene rearrangements involving *BCL2*, *BCL6*, *MYC*, and *MALT1*. Our study demonstrated that an extensive IHC study is preferred for adequate diagnosis and classification of SRCL.

2 Materials and methods

Our pathology department archives from 1988 to 2016 for “signet ring cell lymphoma, lymphoma with signet ring cells” were searched. Clinical information and the results of flow cytometric analysis were collected from the medical records. Five cases from 4 patients were identified. Two additional cases were contributed by the collaborating colleagues. This study was approved by the institutional review board of our institute.

Immunohistochemical stains were performed at the referring institutions or in the laboratory at Indiana University Health Pathology Laboratory using the prediluted, ready-to-use antibodies (CD3, clone IR503; CD20, IR604; CD10, IR648; BCL2, IR614; BCL6, IR625; Ki-67, IR626; MUM-1, IR644) from Dako with a Dako Autostainer Plus instrument following the manufacturer's protocols [18].

This is the author's manuscript of the article published in final edited form as:
Zhang, S., Sun, J., Fang, Y., Nassiri, M., Liu, L., Zhou, J., ... & Vance, G. H. (2017). Signet-ring cell lymphoma: clinicopathologic, immunohistochemical, and fluorescence in situ hybridization studies of 7 cases. Annals of diagnostic pathology, 26, 38-42. https://doi.org/10.1016/j.anndiagpath.2016.10.002

Fluorescence in situ hybridization studies were performed using 4-μm tumor tissue sections that were mounted on positively charged glass slides. An accompanying hematoxylin and eosin slide was reviewed to determine tumor location. Slides were baked at 65°C ± 5°C for 1 hour, deparaffinized in xylene at room temperature, and washed in 100% ethanol and air-dried. Pretreatment included exposure to 0.2 N HCl, followed by 1 M sodium thiocyanate and pepsin at 37°C to digest proteins in the tissue sample. After pepsin treatment, slides were viewed with phase microscopy to ensure adequate digestion of the tumor tissue. Denaturation, ethanol dehydration, and hybridization with the LSI MYC Dual Color, Break Apart Rearrangement; LSI IGH/MYC, CEP 8 Tri-color Dual Fusion Translocation; LSI BCL6 Break Apart and LSI IGH/BCL2 Dual Color, Dual Fusion Translocation (Abbott Molecular, Des Plaines, IL) probes were carried out as per manufacturer's instructions. Cells were counterstained with DAPI and observed under a Leica DMRXA2 fluorescence microscope. Two hundred cells were analyzed by 2 readers (100 cells apiece) for each of the 3 probes. Scoring criteria included the selection of single nuclei with representation of at least 1 signal for each color/probe/nucleus.

3 Results

3.1 Clinical features

A total of 7 cases from 6 patients (3 women and 3 men) with slides available for review and material for FISH study were included in our study. The patients ranged in age from 31 to 75 years (average 60.3 years; Table 1). Five of the cases were FL including 1 case with concurrent DLBCL. The initial lesions involved lymph nodes, tonsil, parotid gland, and breast tissue. One patient (cases 2 and 3) initially presented with breast mass with diagnosis of DLBCL with signet-ring cell morphology. Four and half years later, the patient developed extensive lymphadenopathy and a thigh mass which was subsequently diagnosed as low-grade FL with signet-ring cell morphology.

Table 1 Summary of clinicopathologic features and follow-up

alt-text: Table 1

Case	Age (y)	Sex	Site of biopsy	Stage	Diagnosis	Treatment	Follow-up
1	31	F	Parotid, LN	II	DLBCL, FL 3	R-CHOP ×6	Local recurrence at 20 mo
2	71	F	Breast	III	DLBCL	Chemotherapy ×8	Dead 4.5 y
3	75	F	Thigh mass	Unknown	FL, low-grade	Unknown	Dead, 4 mo later
4	60	M	Submandibular LN	Unknown	FL, 1-2	Resection + radiation	Free of disease
5	60	M	LN	Unknown	FL 3	Unknown	Not available
6	52	F	Tonsil	Unknown	DLBCL, MZL	Unknown	Not available
7	73	M	LN	IIIA	FL, 1-2	Observation	Stable

Abbreviations: LN, lymph node; MZL, marginal zone lymphoma; R-CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone, and rituximab.

3.2 Histopathologic features

Microscopic examination demonstrated all cases to contain numerous signet-ring cells with abundant clear or vacuolated cytoplasm (cases 1-5), or abundant eosinophilic, Russell body-like cytoplasm (cases 6 and 7). As shown in Fig. 1, the breast mass needle biopsy sample (case 2) showed diffuse proliferation of large, atypical polymorphic cells with irregular nuclear contours, dispersed or vesicular chromatin, one to several distinct nucleoli, and abundant clear cytoplasm. Mitotic figures and apoptotic bodies were easy to find. The biopsy (Fig. 2) of the left thigh mass (case 3) from the same patient 4 years later showed nodular proliferation of predominantly small atypical lymphoid cells with irregular nuclear contours, dispersed chromatin, inconspicuous nucleoli, and abundant clear cytoplasm in a background of delicate fibrosis and vascular proliferation. Few scattered large atypical lymphoid cells with vesicular chromatin and multiple nuclear membrane–associated nucleoli are present. In case 6, the right tonsil removed from a 53-year-old woman showed 3 areas with different morphology: 1 portion (not shown) with preserved follicular lymphoid architecture and 1 portion (Fig. 3A) with vaguely nodular proliferation of predominantly small atypical lymphocytes with condensed chromatin, slightly irregular nuclear contours, inconspicuous nucleoli, and moderately abundant clear cytoplasm. Scattered large atypical lymphoid cells and aggregates of plasma cells are noted. The third portion (Fig. 3B) contains sheets of large atypical lymphoid cells with vesicular chromatin and multiple inconspicuous nucleoli admixed with frequent large signet-ring-like cells containing round pink globules, resembling very large Russell bodies. Similar to case 6, case 7 (Fig. 4) showed nodular and diffuse proliferation of predominantly small atypical lymphoid cells with abundant eosinophilic cytoplasm pushing the nuclei to the periphery of cells.

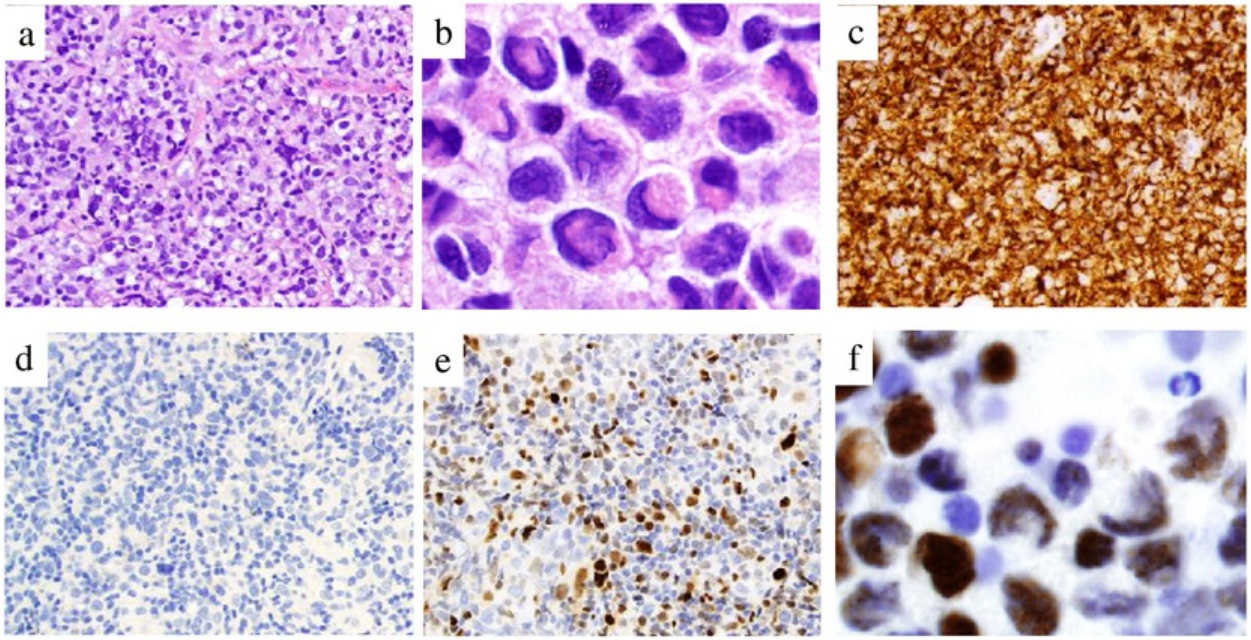

Fig. 1 Diffuse large B-cell lymphoma with signet-ring morphology (case 2). (A and B) Hematoxylin and eosin, original magnifications ×20 (A) and ×100 (B). (C-F) Immunohistochemistry: (C) CD20 (×20), (D) CD21 (×20), (E) MUM-1 (×20), and (F) BCL-6 (×100).

alt-text: Fig. 1

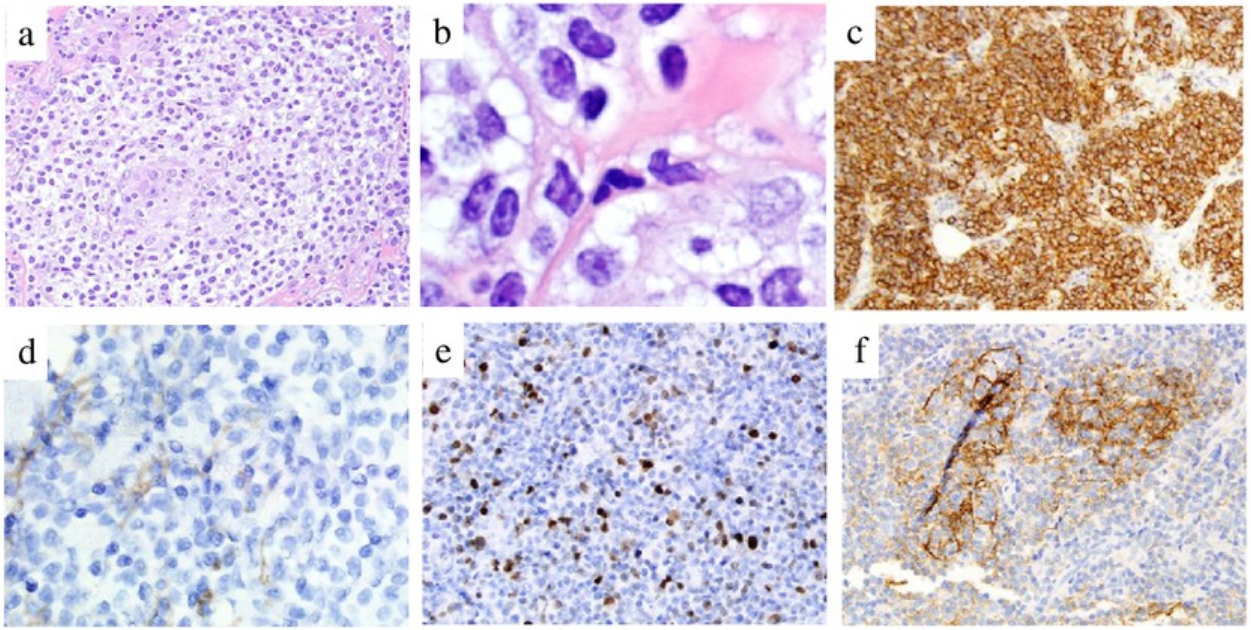

Fig. 2 Follicular lymphoma with signet-ring morphology (case 3). (A and B) Hematoxylin and eosin, original magnifications ×20 (A) and ×100 (B). (C-F) Immunohistochemistry (×20): (C) CD20, (D) CD10, (F) MUM-1, and (F) CD21.

alt-text: Fig. 2

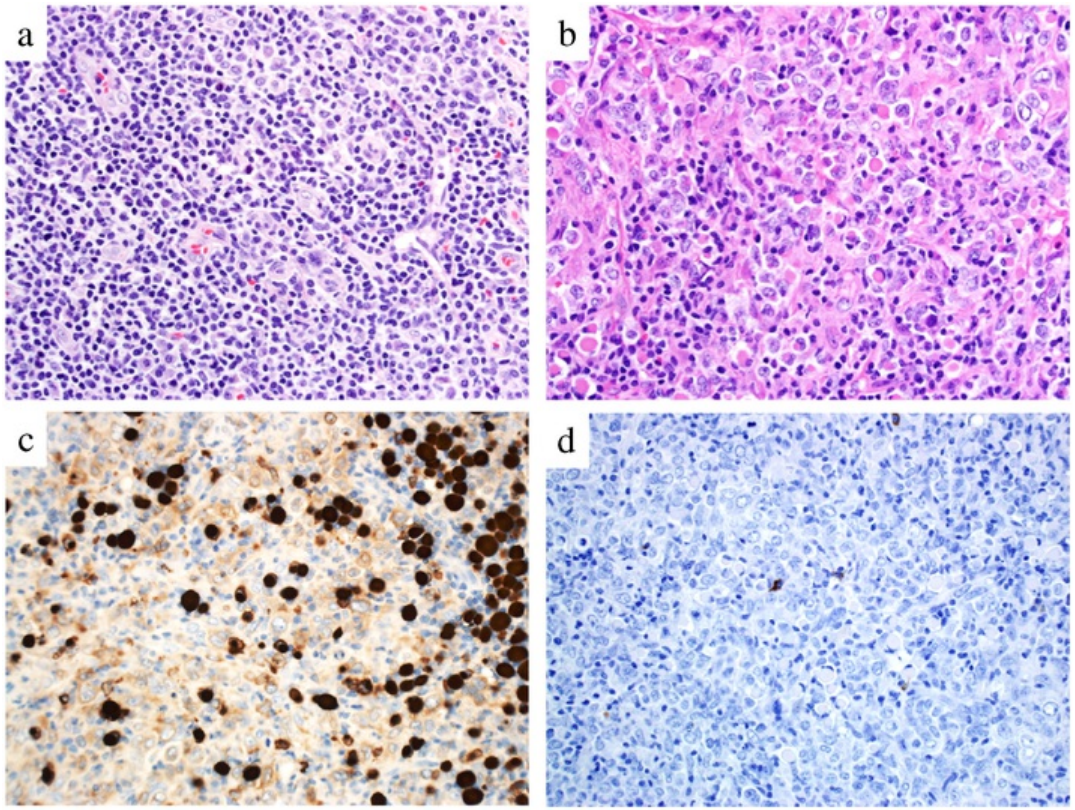

Fig. 3 (A) Low-grade lymphoma (case 6; hematoxylin and eosin, original magnification ×50). (B-D) Large B-cell lymphoma with signet-ring morphology (hematoxylin and eosin, ×50 [B]; immunohistochemistry, ×40 [C-D]). κ (F) and λ (D).

alt-text: Fig. 3

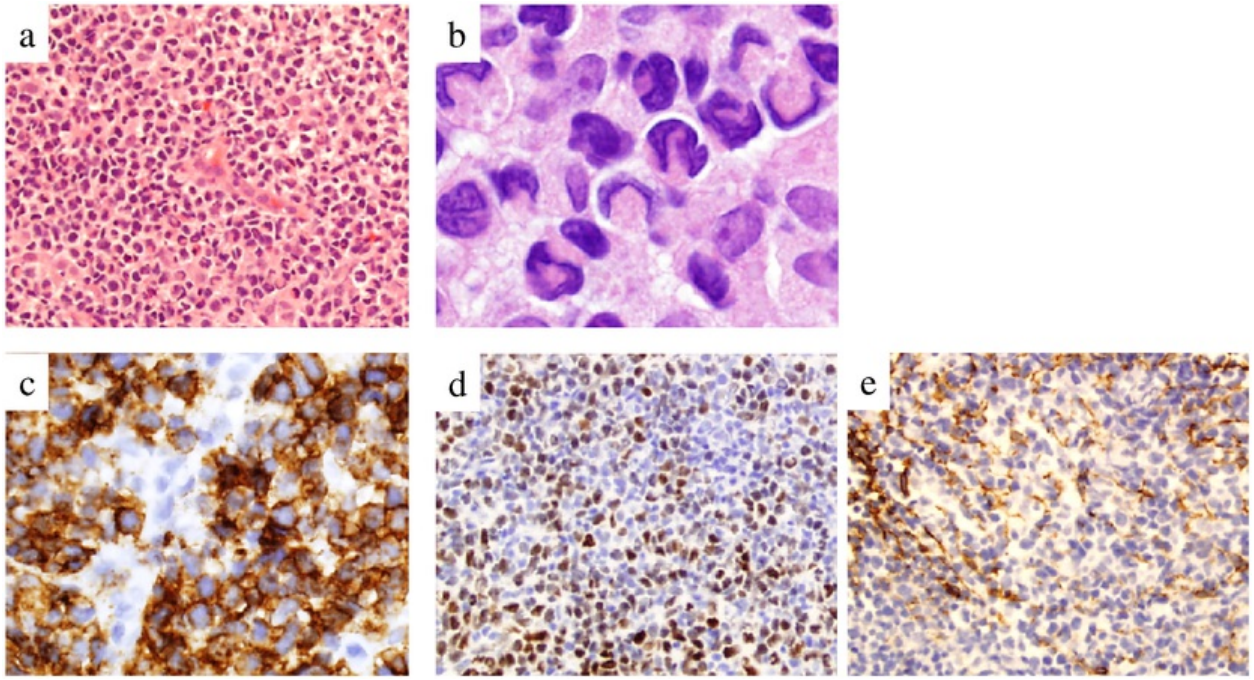

Fig. 4 Follicular lymphoma with signet-ring morphology (case 7). (A and B) Hematoxylin and eosin, original magnifications ×20 (A) and ×100 (B). (C-E) Immunohistochemistry, ×20: CD20 (C), BCL-6 (D), and CD21 (E).

alt-text: Fig. 4

3.3 Immunohistochemical findings

The results of IHC stains of the 7 cases are summarized in Table 2. The signet-ring cells in all 7 cases were positive for CD20 and also positive for PAX-5 in all the 6 cases stained for PAX-5, confirming that they were of B-cell lineage. There were preserved follicular dendritic cell (FDC) meshworks as demonstrated by positive CD21 and/or CD23 stains in the FL cases (cases 1, 3, 4, 5, and 7). On the contrary, there were no demonstrable FDC meshworks in cases with DLBCL (case 2) or in area with DLBCL (case 6). Interestingly, 2 of the 3 cases with low-grade FL were negative for CD10 but positive for BCL6 and/or MUM-1. The SRCL cells in cases 2 and 3 showed essentially identical immunophenotype, suggesting that they might be clonally related. In case 6, the signet-ring cells were positive for cytoplasmic κ and negative for λ (Fig. 3C and D). Furthermore, IHC (data not shown) showed that these signet-ring cells were also positive for CD138, IgD, and MUM-1, and negative for IgM, IgG, IgA, and cytokeratin (AE1/AE3). Although sharing similar morphology with case 6, the signet-ring cells in case 7 (data not shown) were negative for CD138, IgM, IgD, IgG, IgA, and κ and λ light chain. None of the neoplastic cells were positive for mantle cell lymphoma markers cyclin D1 and SOX11.

Table 2 Summary of IHC stains

alt-text: Table 2

Case	CD3	CD5	CD20	PAX5	CD23	CD10	BCL2	BCL6	MUM1	CD21	Cyclin D1	SOX11	Ki-67
1	–	–	+	+	+, *	+	+	+	–	*	–	–	70%
2	–	–	+	+	–	–	+	+/–	+	*, focal	–	–	10%-40%
3	–	–	+	+	–	–	+	+/–	+/–	*	–	–	20%
4	–	–	+	+	–, *	–	+	+	–	–	–	–	20%-30%
5	–	–	+	+	–, *	+	+	–	–	–	–	–	20%
6	–	ND	+	+	ND	–	+	+	+	*, focal	–	ND	30%-40%

7	–	ND	+	ND	ND	+	+	+	–	*	–	ND	10%

Abbreviations: *, positive for FDC meshwork; ND, not done.

3.4 Fluorescence in situ hybridization findings

As our index case (case 6) showed complex changes including *BCL6* rearrangement seen in approximately 30% of cells analyzed in addition to increased copy numbers of *MYC* and *MALT1*, FISH studies for *BCL6*, *MYC*, *MALT1*, and *BCL2* (*IGH/BCL2*) rearrangements were performed on the other 6 cases except that *MYC* was not performed in case 7 (Table 3). Surprisingly, an *IGH/BCL2* translocation was detected in only 1 case (case 5) of the 5 FL cases (cases 1, 3, 4, 5, and 7) including none of the 3 low-grade FL cases (cases 3, 4, and 7). There was also increased *MALT1* copy number in case 7. Case 3 showed copy number changes for both *BCL6* and *MYC*. No abnormalities were detected in the remainder of the cases.

Table 3 Summary of FISH study

alt-text: Table 3

Case	IGH/BCL2	BCL6	MYC	MALT1	API2/MALT1
1	–	–	–	–	ND
2	–	–	–	–	ND
3	–	3 copies	1 copy	–	–
4	–	–	–	–	ND
5	+	–	–	–	ND
6	–	30% R	3-7 copies	3-6 copies	ND
7	–	–	ND	3-4 copies	–

Abbreviations: ND, not done; R, rearranged.

3.5 Clinical follow-up

Clinical treatment and follow-up were available in 4 of the 6 patients. The patient (case 1) with parotid DLBCL and high-grade FL was treated with cyclophosphamide, doxorubicin, vincristine, prednisone, and rituximab for 6 cycles. She obtained initial remission; however, a local recurrence was detected at 20 months after her initial diagnosis. She was lost to further follow-up. The patients (cases 2 and 3) were treated with chemotherapy (unknown regimen) for 8 cycles after her initial diagnosis of breast DLBCL and achieved remission till 4 years later with a diagnosis of low-grade FL in the thigh mass. The patient died 4 months later with a total survival of 4.5 years from her initial diagnosis of DLBCL. The patient (case 4) with low-grade FL received local resection and radiation (dosage unknown). He was free of disease at the last follow-up approximately 5 years after his initial diagnosis. There was no information available about the clinical treatment and follow-up in cases 5 and 6. Case 7 was a new diagnosis with no specific treatment currently.

4 Discussion

Signet-ring cell lymphoma has been originally reported as a morphologic variant of FL and subsequently reported in other types of NHL, mostly as single-case report. The current study represents the first series with comprehensive studies including detailed IHC stains and FISH studies. Consistent with the literature, SRCL is most commonly seen in FL (5/7). Although BCL2 was expressed in all 5 FLs, only 1 case (case 5) had *IGH/BCL2* translocation, indicating other mechanisms involved in inducing abnormal BCL2 expression by IHC. BCL6 was expressed in all 7 cases (focal positivity in cases 2 and 3), with FISH analyses showing increased *BCL6* copy number (3 copies) in one case and *BCL6* rearrangement in another one. *BCL6* was interpreted as normal in the other 5 cases by FISH. Interestingly, increased copy numbers of *MYC* and *MALT1* in addition to a *BCL6* rearrangement were also present in case 6, which suggest that *MALT1* may be involved in the MZL component, whereas *BCL6* and *MYC* abnormalities may contribute to the large cell transformation (DLBCL) seen in this case. These findings suggest that although all 7 cases contain signet-ring cell morphology, accurate lymphoma diagnosis and classification require detailed IHC study.

Kim et al [1] first reported 7 SRCL cases from predominant lymph nodes almost 40 years ago. Two types of signet-ring cells were described: one with clear vacuolated cytoplasm containing IgG and the other with eosinophilic cytoplasm containing Russell body-like immunoglobulin IgM (κ). They were considered as a rare morphologic variant of FL. Ultrastructural study by electron microscopy and IHC stains suggested that the clear vacuoles seen in both B- and T-cell SRCL were

related to aberrant membrane recycling, whereas the Russell body-like inclusions seen only in B-cell NHLs were related to abnormal secretion and accumulation of immunoglobulin [1,19,20]. Our cases 6 and 7 provided evidence that immunoglobulins other than IgM (κ; case 6) or other nonimmunoglobulin proteins (case 7) may also accumulate inside the cytoplasm, contributing to the prominent signet-ring cell morphology.

To date, more than 50 SRCL cases have been reported in both B- and T-cell NHL and plasma cell myeloma, involving predominantly lymph nodes and less frequently extranodal tissues. Our series expands the current literature of cases of SRCL, with emphasis that SRCL may be seen in a broad spectrum of B-cell lymphoma including FL, MZL, and DLBCL. Practically, it is important for pathologists, particularly general surgical pathologists, to be aware of this rare morphologic presentation of lymphoma, especially when it involves an extranodal tissue as signet-ring cell adenocarcinoma or melanoma may be the first differential diagnosis. Lymphoid marker such as CD3 and CD20 should be performed first before other pan-cytokeratin and melanocytic markers, especially when only limited tissue material is available from a needle biopsy. However, once a lymphoma is suggested from initial IHC study, a more extended IHC study should be performed to properly classify the lymphoma according to current World Health Organization classification.

It is generally accepted that prognosis of SRCL is related to its underlying type of lymphoma. Whether the presence of signet-ring cell morphology may impact its prognosis compared with its counterpart without signet-ring cell morphology is difficult to assess due to rarity of SRCL. In our case series, patient (case 2) survived 4.5 years after her initial diagnosis of DLBCL. This would be considered a much better survival when her initial DLBCL might represent a transformation from her (unidentified) underlying low-grade FL, as FL transformation is associated with a median overall survival of less than 2 years. Another patient (case 1) with both DLBCL and high-grade FL achieved remission after chemotherapy and had only local recurrence at 20 months after initial diagnosis.

In conclusion, we described 7 SRCL cases which represent 3 types of B-cell NHL. The FL with signet-ring cell morphology tends to lack *IGH/BCL2* translocation, although *BCL2* is expressed in the FL cells. We recommend an extended IHC study to include minimally CD3, CD20, CD5, CD10, CD21, CD23, BCL2, BCL6, and cyclin D1 for correct diagnosis and classification of SRCL.

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