A rare case of signet ring cell lymphoma: Diagnosis aided by immunofluorescent staining

ABSTRACT
Signet ring cell lymphomas are the proliferations of malignant lymphoid cells containing cytoplasmic vacuoles or globules which displace the nuclei, imparting it a signet ring appearance. This rare tumor is a variant of non-Hodgkin lymphoma. Signet ring appearance is due to cytoplasmic accumulation of immunoglobulin or vacuoles derived from multivesicular bodies. These cells, particularly with cytoplasmic vacuoles, may be mistaken for adenocarcinoma cells. We are presenting one such case where immunofluorescence helped us to demonstrate the immunoglobulins on fine needle aspiration smears. This is an innovative technique and has not been reported earlier. Our aim of presenting this case is to review the awareness of this rare lymphoma among pathologists to give due consideration for avoiding inappropriate investigations and treatment.

Key words: Immunofluorescence; lymphoma; signet ring cell

Introduction
Signet ring cell lymphoma (SRCL) are proliferations of malignant lymphoid cells containing cytoplasmic inclusions or vacuoles that displace the nucleus, imparting it a “signet ring” appearance. It is a rare morphological variant of non-Hodgkin lymphoma (NHL), which can be confused with other more common tumors such as signet ring cell carcinomas, especially in cytological preparations and bone marrow biopsies. Though originally described as a variant of follicular lymphoma, signet ring morphology has been seen in other types of NHL as well. The signet cell appearance in these cases may be due to cytoplasmic accumulation of immunoglobulin (Ig) or vacuoles derived from multivesicular bodies (MVBs). However these morphological features in lymphomas are very rare and prompted us to report one such case where immunofluorescence (IF) helped us to demonstrate IgG in the cytoplasm of these cells. We have not come across any other instance in literature where IF was used for this purpose in SRCL.

Case Report
A 60-year-old male presented with a 2-month history of pain and fullness in the abdomen, breathlessness, and weight loss. On palpation, left supraclavicular and cervical lymphadenopathy was revealed. A single, large, firm lump with nodular surface was felt in the umbilical region. It was extending into epigastrium, lumbar, and hypogastric regions.
Radiological investigations showed bilateral plural effusion and ascitis with extensive para-aortic, retroperitoneal, celiac, portal and mesenteric nodal masses. Patient had moderate anemia and raised erythrocyte sedimentation rate (ESR). Clinical impression was of either a disseminated carcinoma or malignant lymphoma.

Fine needle aspiration smears of cervical lymph node predominantly revealed a monotonous population of medium sized lymphoid cells with scanty to moderate cytoplasm and cleaved and noncleaved nuclei showing inconspicuous nucleoli. Scattered among these were larger cells with prominent cytoplasmic vacuoles and a typical signet ring cell morphology with eccentrically placed flattened or crescentic nuclei. The background showed lymphoglandular bodies [Figure 1a].

The histochemical stains for mucin (periodic acid Schiff and mucicarmine) were negative. To demonstrate Ig in the cytoplasm of these cells, IF technique was used. We re-aspirated the cervical lymph nodes, and cell suspensions were prepared in phosphate buffer saline (PBS) in two test tubes. From the first tube, the cells were washed twice and the smears were prepared from the deposit. From the second tube, the cell pellet was transferred to frozen section medium and cell block was prepared. The smears and cell block sections both were stained for IgG and IgM using the direct IF technique. To our surprise, the signet ring cells gave brilliant fluorescence for IgG antibody [Figure 1b]. No reaction could be seen with the IgM antibody. The IF was better demonstrated on direct smears rather than sections.

Pleural fluid cytology also showed typical signet ring cells in addition to lymphocytes and mesothelial cells. These signet ring cells showed negativity for the mesothelial marker HBME1 on immunocytochemistry [Figure 1c].

The lymph node biopsy showed classical follicular lymphoma with some diffuse areas [Figure 1d]. The signet ring cells were seen mostly in diffuse areas and interfollicular areas [Figure 2a, arrow]. There were no plasmacytoid cells, cells with eosinophilic Russel body-like inclusions or large blast cells. Necrosis was absent.

Immunohistochemistry demonstrated positivity for CD 10, CD 20, CD 45, and negative for T-cell markers such as CD 3 and CD 5, as well as pan-cytokeratin [Figure 2b-d].

Bone marrow biopsy sections also showed presence of signet ring cells. Considering the secreting nature of the neoplastic cells, the pleural fluid and serum were subjected to electrophoresis on cellulose acetate membrane at pH 8.6. Both showed prominent monoclonal band in gamma region.

In consideration of all these findings, a final diagnosis of B-cell follicular lymphoma Grade I Signet ring cell type (stage IV) was offered. The patient was treated with appropriate chemotherapy and the nodes regressed very rapidly. The pleural effusion persisted comparatively longer and was treated with repeated tapping. The patient showed significant improvement, however, after few months the...
Discussion

The term SRCL was first coined by Kim et al. in 1978. Initially, it was thought to be a variant of follicular cell lymphoma. However, subsequently, various workers described similar morphology in T-cell, immunoblastic, Burkitt-like, MALT-type, and small and large cell lymphomas. The nature of the vacuoles or cytoplasmic inclusion is yet under debate, and may be IgG (in centrocytic lymphoma) or IgM, forming Russel body-like eosinophilic globules (immunoblastic and large cell lymphoma) or membrane bound spaces derived from multivesicular bodies (EM observation) especially in T-cell SRCL. However, the morphology is rare and may cause confusion in diagnosis in certain situations. In the metastatic lesions and extranodal sites such as the skin, bone marrow, stomach, thyroid, salivary gland and ovary primary suspicion may be of adenocarcinoma, melanoma or liposarcoma.

In addition to negative stains for mucin, the absence of clumping of cells and the fact that in most secondary deposits the aspirations smears are mostly entirely neoplastic epithelial cells, rarely having a predominantly lymphoid background, are helpful pointers for differential diagnosis.

Except in cases with Russell body-like inclusions, where IgM could be unequivocally demonstrated, the immunocytochemical staining for IgG has not always been convincing even in B cell lymphomas. In this context, the IF technique using fresh washed cytological smears may be rewarding, as in our case. Incidentally, there are no previous reports on the use of IF for demonstration of IgG in SRCL. Further proof of the secretary nature of the neoplastic cells in our case was available by demonstration of monoclonal band in gamma region on electrophoresis of serum and pleural fluid. This has also been not reported by others.

Mesothelial cells in pleural fluid often show signet ring cell morphology. We could distinguish neoplastic cells because of the absence of mesothelial markers [Figure 1c, inset]. Recently one large case series of SRCL comprising seven cases with predominant emphasis on cytological features was published. The authors of the case series emphasized distinguishing features of SRCL from melanoma, adenocarcinoma, liposarcoma as well as benign histiocytic proliferations.

Our aim of presenting this case is to review the awareness of this rare lymphoma among the pathologists to give due consideration for avoiding inappropriate investigations and treatment. Our new innovative immunofluorescence technique on fine needle aspiration samples can be utilized as a rapid tool to demonstrate immunoglobulins in these cells in conjunction with the histochemical stains.

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Conflicts of interest
There are no conflicts of interest.

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